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AgriSciences**

**Acoustic fingerprinting in Black-and-white Laughingthrush  
(*Garrulax bicolor*): non-invasive monitoring of endangered  
species**

Diploma thesis

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## **Declaration**

I hereby declare that this diploma thesis on the theme: Acoustic fingerprinting in Black-and-white Laughingthrush (*Garrulax bicolor*): non-invasive monitoring of endangered species was elaborated independently and is based only on my own knowledge, consultations with my supervisor and literary resources cited in attached bibliography.

In Prague 24th of April 2015

Bc. Barbora Vališová

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## **Abstract**

### **Acoustic fingerprinting in Black-and-white Laughingthrush (*Garrulax bicolor*): non-invasive monitoring of endangered species**

Black-and-white Laughingthrush is a difficult species to survey according to its hidden way of life and declining trend in its population size in the montane forests of Sumatra. According to its taxonomic history (recognized as a separate species in 2006), there are few information known about the accurate population size and behavioural ecology. This research presents the first bioacoustic study of the Black-and-white Laughingthrush. The aim was to determine whether some vocalizations of this species contain individually specific features of vocal individuality, which could be used as a tool for further non-invasive monitoring of the species. I analysed the basic song types collected during the nesting season of 24 captive individuals in Cikananga Wildlife Rescue Center (West Java).

Four different vocalization types were identified in vocal repertoire of Black-and-white Laughingthrush: twitter, trill, contact song and duet. Significant differences between individuals occurred mainly in frequency parameters in each call type. Potential for individuality coding (PIC) was computed by comparing the coefficients of variation among individuals and the mean coefficients of variation between individuals for the parameters of each type of vocalization. The PIC value was higher than one in all parameters in twitter and trill, except one in all parameters of contact song and in 22 from 32 variables in duet. Resulting model of DFA included seven from 32 measured parameters (Wilk. Lambda = 0,146). Overall classification result showed 75,8% correct assignment of duets into the correct pair. I can conclude that Black-and-white Laughingthrush vocalizations contain highly individually specific features (based on PIC) and especially duet as a long-distance signal represent be the most interesting candidate for vocal tagging.

#### **Key words:**

Black-and-white Laughingthrush, *Garrulax bicolor*, vocal individuality, individual variability, non-invasive monitoring

## **Abstrakt**

### **Akustický fingerprinting u sojkovce dvoubarvého (*Garrulax bicolor*): neinvazivní monitoring ohroženého druhu**

Výzkum a pozorování sojkovce dvoubarvého je velice obtížný kvůli jeho skrytému způsobu života a jeho snižujícímu se populačnímu trendu v horských lesích Sumatry. Vzhledem k jeho taxonomické historii (byl uznán jako samostatný poddruh v roce 2006) je známo velice málo informací o přesné velikosti populace a behaviorální ekologii tohoto druhu. Tento výzkum představuje první bioakustickou studii sojkovce dvoubarvého. Cílem bylo určit, zda nějaká vokalizace tohoto druhu zahrnuje přítomnost individuálně specifických rysů, které by mohly být použity jako nástroj k jeho dalšímu neinvazivnímu pozorování. Analyzovala jsem základní typy hlasů shromážděných během hnízdního období od 24 jedinců chovaných v zajetí v záchraném centru Cikananga Wildlife Rescue Center (západní Jáva).

V hlasovém repertoáru sojkovce dvoubarvého byly identifikovány čtyři různé typy vokalizace: štěbetání, trilek, kontaktní zpěv a duet. Signifikantní rozdíly mezi jedinci byly především ve frekvenčních parametrech každého hlasu. Potenciál pro kódování individuality (PIC) byl vypočítán srovnáním variačních koeficientů mezi jedinci a průměrem variačních koeficientů uvnitř jedinců pro parametry všech typů vokalizace. Hodnota PIC byla větší než jedna ve všech parametrech štěbetání a trilku, kromě jednoho ve všech parametrech kontaktního zpěvu a ve 22 ze 32 parametrech duetu. Výsledný model DFA zahrnul sedm ze 32 měřených parametrů (Wilk. Lambda = 0,146). Celkový klasifikační výsledek určil 75,8% správně přidělených duetů k náležitému páru. Mohu konstatovat, že vokalizace sojkovce dvoubarvého obsahují vysoce individuálně specifické rysy (na základě PIC) a především duet, jako akustický signál na velkou vzdálenost, představuje nejzajímavějšího kandidáta pro vokální značení.

#### **Klíčová slova:**

sojkovec dvoubarvý, *Garrulax bicolor*, hlasová individualita, individuální variabilita, neinvazivní monitoring

## Contents

1	Introduction .....	12
2	Literature Review .....	14
2.1	Black-and-white Laughingthrush ( <i>Garrulax bicolor</i> ).....	14
	Taxonomy.....	14
	Morphology .....	14
	Behaviour .....	15
	Habitat and Distribution .....	16
	Population.....	17
	Threats .....	17
	Conservation.....	18
2.2	Monitoring of endangered species .....	18
	Non-invasive acoustic monitoring.....	18
2.3	Acoustic Communication.....	20
2.4	Individual recognition .....	21
2.5	Vocal Repertoire .....	22
	Duet .....	23
3	Aims of the thesis .....	24
4	Material and Methods.....	25
4.1	Study Site .....	25
4.2	Study Birds.....	26
4.3	Recording .....	26
4.4	Sampling .....	28
4.5	Acoustic analysis.....	29
4.6	Statistical Analysis .....	32
5	Results .....	34
5.1	Repertoire.....	34
	Twitter .....	34
	Trill.....	38
	Contact song .....	42
	Duet .....	46
5.2	Social type and context .....	50
	Twitter .....	50
	Trill.....	51
	Contact song .....	52

Duet .....	53
5.3 Sex and other categories.....	55
Contact song .....	55
Duet .....	56
5.4 Individual variability .....	58
5.4.1 Univariate statistics and Potential of Individuality Coding (PIC) .....	58
Twitter .....	58
Trill .....	59
Contact song .....	60
Duet .....	61
5.4.2 Nonparametric test of variation coefficients.....	63
5.4.3 Discriminant Function Analysis (DFA).....	64
6 Discussion.....	67
Repertoire.....	67
Influence of social type, context and sex .....	68
Social type .....	68
Context .....	68
Sex .....	68
Individual variability .....	69
Differences between individuals .....	69
Potential for Individuality Coding (PIC).....	70
DFA .....	71
Monitoring and conservation .....	72
7 Conclusions .....	74
8 References .....	75

## List of figures

Fig. 1. Morphology of Black-and-white Laughingthrush (© Barbora Vališová).....	15
Fig. 2. Head of Black-and-white Laughingthrush with the specific signs of its morphology (Collar, 2006).....	15
Fig. 3. The map of distribution of Black-and-white Laughingthrush in Sumatra, Indonesia (BirdLife International, 2015).....	16
Fig. 4. Signalling system in birds including sender, signal and receiver (Gillam, 2011)....	21
Fig. 5. The location of Cikananga Wildlife Rescue Center in West Java, Indonesia (Cikananga Wildlife Center, 2015).....	25
Fig. 6. The manually measured parameters in spectrogram of duet: Cadency, Duration of the duet, Duration Female, Duration M1, Duration M2, Interval M1-M2, Duration of twitter part of male, F trill cadency, High Frequency and Low Frequency. In this specific duet the duration of the duet was equal to duration of the female call, the cadency was FMFMF and the number of the elements of male part was 11.....	32
Fig. 7. Spectrogram and waveform of twitter.....	35
Fig. 8. Spectrogram and waveform of trill.....	39
Fig. 9. Spectrogram and waveform of contact song - male.....	43
Fig. 10. Spectrogram and waveform of contact song - female.....	43
Fig. 11. Spectrogram and waveform of duet.....	47
Fig. 12. Influence of social type on twitter in the parameter of Frequency 95% (Hz) (the frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy in the selection, the summed energy has to exceed 95% of the total energy). The significant differences were between pair and two males, and between pair and female in separate enclosure (* $p < 0,05$ , ** $p < 0,01$ ).....	50
Fig. 13. Influence of social type on trill in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal). The most significant difference was between pair and female (***) $p < 0,001$ .....	51
Fig. 14. Influence of context on trill in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal).....	52



Fig. 15. Influence of social type and context on contact song in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal). The most significant differences were between pair and female housed alone in standard situation (\*\*p < 0,01) and in other situation, when the keeper approached to the enclosures or when the individuals responded to each other (\*p < 0,05). The significant difference were also between female and male (both in separate enclosures) in standard situation (\*p < 0,05). 53

Fig. 16. Influence of social type on frequency range (Hz) (the difference between the highest and lowest frequency) in duet. The significant influence was between pair and pair with hatched chicks and between female and male in separate enclosures in comparison with pair with hatched chicks (\* p < 0,05, \*\*\* p < 0,001). .....54

Fig. 17. Influence of context on duet in the parameter Dur 90% (s) (the difference between the 5% and 95% times). The most significant differences were between approach and answer and between approach and standard (\*\* p < 0,01, \*\*\* p < 0,001). .....55

Fig. 18. The difference between female (F) and male (M) in the parameter 1st Quartile Frequency (Hz) (the frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the selection, the summed energy has to exceed 25% of the total energy) of contact song. ....56

Fig. 19. Influence of the sex of individual who initiated duet on the Aggregate Entropy (the aggregate entropy measures the disorder in a sound by analysing the energy distribution within a selection, higher entropy values correspond to greater disorder in the sound whereas a pure tone would have zero entropy) of duet. The most significant difference was between MF (when the individuals started the duet together) and F (when the female started to sing duet) (\*\*p < 0,01) and between MF and M (when the male started to sing duet) (\*\*p < 0,01). .....57

Fig. 20. Influence of the sex of individual who initiated duet on the duration of the 1st male part (s) of duet in the standard context situation and when the individuals were affected by approach of keeper or when they reacted to each other. Differences of sex of the individual who started to sing duet were the most significant between female and male in standard situation (\*\*p < 0,01) and in the other situations (approach, answer) (\*p < 0,05), and between male and both sex in standard situation (\*p < 0,05). .....58

Fig. 21. Difference between pairs in the duration (s) of duet. ....63

Fig. 22. Inter- and intra-individual mean coefficients of variation for each call type of the repertoire of Black-and-white Laughingthrush: Twitter, Trill, Song and Duet. ....63

Fig. 23. Plot of five pair's duets of Black-and-white Laughingthrush on the space of the first two discriminant functions and the spectrogram of each pair's duet. .... 66

## List of tables

Tab. 1. Recording Cycle 1: Number of individuals in each building and number of enclosures, individuals, pairs and separate individuals recorded. ....	27
Tab. 2. Recording Session 2: Number of individuals in each building and number of enclosures, individuals, pairs and separate individuals recorded. ....	27
Tab. 3. Recording Session 1: Marked enclosure from 1 to 14, with their inhabitants and its social type (pair - 1+1, male alone - 1+0, female alone - 0+1, two males together - 2+0, two males and one female - 2+1, unknown sex - ?) .....	28
Tab. 4. Recording Session 2: Marked enclosure from 1 to 9, with their inhabitants and its social type (pair - 1+1, pair with hatched chicks - 1+1+).....	29
Tab. 5. Description of measured parameters chosen for the acoustic analysis of Black-and-white Laughingthrush in the RavenPro v. 1.4 (Charif et al., 2010).....	30
Tab. 6. Description of manually measured parameters chosen for the acoustic analysis of Black-and-white Laughingthrush. ....	31
Tab. 7. The summary of call types of Black-and-white Laughingthrush: number of each call performed, number of individuals who expressed it and number of males and females who expressed it, and total number of recorded individuals, recorded males, recorded females and total number of recorded vocalizations. ....	34
Tab. 8. The description of the twitter: representation of sex and context.....	35
Tab. 9. Descriptive statistic of the twitter: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.....	35
Tab. 10. Description of measured acoustic parameters of twitter used for the statistical analysis.....	36
Tab. 11. Correlations of measured parameters of twitter, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.....	37
Tab. 12. The description of the trill: representation of sex and context. ....	38
Tab. 13. Descriptive statistic of the trill: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.....	38

Tab. 14. Description of measured acoustic parameters of trill used for the statistical analysis.....	40
Tab. 15. Correlations of measured acoustic parameters of trill, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.....	41
Tab. 16. The description of the contact song: representation of sex and context.....	42
Tab. 17. Descriptive statistic of the contact song: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.....	42
Tab. 18. Description of measured acoustic parameters of contact song used for the statistical analysis.....	44
Tab. 19. Correlations of measured parameters of contact song, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.....	45
Tab. 20. The description of the duet: representation of sex and context.....	46
Tab. 21. Descriptive statistic of the duet: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded individuals.....	46
Tab. 22. Description of measured acoustic parameters of duet used for the statistical analysis.....	48
Tab. 23. Correlations of measured parameters of duet, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.....	49
Tab. 24. Descriptive statistics (mean $\pm$ SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of twitter and the results of the calculation of their PIC.....	59
Tab. 25. Descriptive statistics (mean $\pm$ SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of trill and the results of the calculation of their PIC.....	60
Tab. 26. Descriptive statistics (mean $\pm$ SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of contact song and the results of the calculation of their PIC.....	61
Tab. 27. Descriptive statistics (mean $\pm$ SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of duet and the results of the calculation of their PIC.....	62

Tab. 28. Comparison of prior probability of correctly matched pairs (expressing duet) by chance with correctly classified pairs after conventional and validated discriminant analysis.....64

Tab. 29. Classification results of discriminant function analysis. The bold values show the correctly classified pairs expressing duet by original discriminant function and after cross-validation. ....65

## 1 Introduction

Black-and-white Laughingthrush is an endemic species of Sumatra who lives in montane habitat of evergreen moist forest (Shepherd, 2010; Collar et al., 2014; BirdLife International, 2015). Laughingthrushes are social and territorial species, who live in flocks containing from four to 30 individuals (Maneekorn, 1987; Round, 2006). In 2006 it has been split from White-crested Laughingthrush according to the differences in morphology (characteristic black-and-white coloration with white head and black body, black beak and black circles around the eyes) and distribution range (Collar, 2006). According to the taxonomic history there are few information known about the behavioural ecology of Black-and-white Laughingthrush as well as about the population size. The recent studies have shown that the population is decreasing and the population size was estimated to 2,500-9,999 individuals in the wild (Collar, 2006; Shepherd, 2007; Shepherd, 2010; Owen et al., 2014; BirdLife International, 2015). The species is mostly threatened by illegal trade due to huge demand for this and many other species in Indonesia and it already caused huge decline of the population size in the wild (Shepherd, 2006; Owen et al., 2014). The harvest quota for that species was established to zero, yet it is taken from the wild and illegally sold in the bird markets (Shepherd, 2007; 2010). Therefore further monitoring of this species is necessary to determine conservation steps for the future protection of the species. Conservation breeding programs for back-up populations have been already settled (Collar et al., 2012).

Monitoring of endangered species can provide new insights into the species and may be used as a control tool whether the conservation efforts are successful (Nichols and Williams, 2006; Morris et al., 2002). Acoustic non-invasive monitoring could bring many advantages and is very suitable for monitoring of species in environments where the animals are difficult to observe. This method is also very useful for monitoring the species which are very sensitive to human approach, which is large when using capture-mark techniques (Grava et al., 2008; Fernández-Juricic et al., 2009; Mennill, 2011; Xia et al., 2012; Linhart et al., 2014). The method of acoustic monitoring assumes that the acoustic communication in birds have some individually distinctive signature.

Birds use acoustic signal to transmit information between the individuals. The information carry messages about sex, mate and parental suitability, and individual identity (Westcott, 2001; Wanker and Fischer, 2001). To recognize individuals is beneficial in

many aspects of social interactions and it stabilizes the social system at all (Wanker et al., 1998; Tibbetts and Dale, 2007). The requirement for individual identity is that the transmitted signals have to be uniform between individuals and have to vary among individuals of the population (Lengagne et al., 1997; Wanker and Fischer, 2001; Lovell and Lein, 2005; Terry et al., 2005; Vignal et al., 2007; Reers et al., 2011).

These facts about the current situation of Black-and-white Laughingthrush led me to examine the vocalization in this species, to determine whether there are clues for individual identity which may be used in the further monitoring and protection of this species.

## 2 Literature Review

### 2.1 Black-and-white Laughingthrush (*Garrulax bicolor*)

#### Taxonomy

Laughingthrushes are passerine birds from the family *Timaliidae*, genus *Garrulax* (Shepherd, 2010). As Collar (2006) described the White-crested Laughingthrush (*Garrulax leucolophus*) consisted of five subspecies: *leucolophus*, *patkaicus*, *belangeri*, *diardi* and *bicolor*. All these subspecies differed from the *leucolophus* in morphology and in the range of distribution. The Black-and-white Laughingthrush (*Garrulax bicolor*) was the only subspecies with the distribution in Sumatra and it had also morphological features, which significantly distinguished it from the other subspecies. The habitat shift between *G. bicolor* and continental taxa was not shown as well. According to these facts Black-and-white Laughingthrush was recognized as a separate species in 2006 by Collar.

#### Morphology

According to Collar (2006) study, Black-and-White Laughingthrush has a blackish body, wings and tail, although juveniles have the mid-belly to vent more white. On the face it has the black round the base of the bill pushing up as a triangle onto the forehead, the white on the side of the forehead moving down in front of the eye. It has a black goggle-like rim around the eye connected with a thick black line of the ear-coverts. The bill and legs are black and the iris is reddish (Collar et al., 2014). The tail is shorter than any of the other races of previous *G. leucolophus* subspecies (Collar, 2006). The size is from 24 to 28 cm and sex are similar (Collar et al., 2014) (Fig.1 and 2).



Fig. 1. Morphology of Black-and-white Laughingthrush (© Barbora Vališová)



Fig. 2. Head of Black-and-white Laughingthrush with the specific signs of its morphology (Collar, 2006).

### **Behaviour**

Most species of laughingthrushes are strongly social birds and in some species is assumed that they are cooperative breeders (Round, 2006). They live in a flock of the same species with four to 30 individuals and with other two to three species. They built nest undergrowth, or in the tree in evergreen forest, mixed deciduous forest, deciduous forest and bamboo forest (Maneekorn, 1987).

There are little known about behaviour of Black-and-white laughingthrush from the wild. According to personal communication with Stephan Bulk (head of the breeding conservation program in Cikananga Wildlife Center, 2013), in captivity the breeding season last all year long. The offspring stay with the parents usually for five weeks, after that they are removed to separate enclosure, because the parents may be aggressive to them



after this period (leaving the young longer with the parents sitting on new eggs are now in progress). They become mature at the age of about six months, but it mostly depends on the surround. It seems that this species is territorial in captivity. If the individuals, especially females, are together with no males around, they are calm. But if there are male around, the females tend to be very aggressive to each other. In the wild it can be expected that group of three to five individuals rear the young and the offspring leave the nest around 12 days according to behavior of *G. leucolophus* (Maneekorn, 1987).

### **Habitat and Distribution**

Black-and-white Laughingthrush is a montane species with the range of 750 – 2000 m and it lives in a broadleaf evergreen forest in the middle and lower storeys (Collar, 2006; Sepherd, 2010; Collar et al., 2014; BirdLife International, 2015).

Most species in the genus *Garrulax* are found in subtropical or tropical areas of southeastern Asia, with few species adapted to temperate or high-altitude climates (Xin et al., 2008). Black-and-white Laughingthrush is an endemic species of Sumatra, Indonesia (Stone, 1902; Collar, 2006; Shepherd, 2010; Lepage and Warnier, 2014) (Fig. 3).



Fig. 3. The map of distribution of Black-and-white Laughingthrush in Sumatra, Indonesia (BirdLife International, 2015).

## **Population**

Black-and-white Laughingthrush was very common in the wild in the past, but recent studies showed, that the population is decreasing (Collar, 2006; Shepherd, 2007; Shepherd, 2010; Owen et al., 2014). It is frequently seen in local wild bird markets in Medan, Sumatra and also in huge bird markets in Jakarta, Java (Shepherd, 2007, Shepherd, 2010). In 2008, Shepherd found out that the Black-and-white Laughingthrush was the most numerous of the laughingthrush species for sale (Sepherd, 2010). However, according to local traders and hunters, the species became rare to find (Shepherd, 2007; Shepherd, 2010). Because of that, the population was estimated to 2,500-9,999 mature individuals (BirdLife International, 2015).

## **Threats**

Indonesia is on the third place with the number of globally threatened bird species in the world (Owen et al., 2014). The island species and the most single-island endemics are at greater risk compared with the species that are more widespread, because they have to face the loss of their habitat at much greater extend. The montane habitat of Sumatra in which Black-and-white Laughingthrush occur was not logged as much as the lowland forests in the past. But as lowland forests disappear, the species is in danger because of habitat loss and for better access of the bird trappers (Shepherd, 2007; Shepherd, 2010). High human population in Indonesia put a lot of pressure on the nature and natural resources. Many people keep birds in the cages and there is a high demand for them, which has the great impact on many species, most on the songbirds. Because of that, many species endemic to Indonesia have already suffered huge declines (Shepherd, 2006; Owen et al., 2014). Trapping for commercial trade already influenced the population of Black-and-white Laughingthrush whose population was significantly reduced (Collar, 2006; Collar et al., 2012; Shepherd, 2007; Shepherd, 2010). Black-and-white Laughingthrush is listed as Vulnerable in the IUCN Red List, but more information about the population size and trends can lead to the reclassification as endangered species (BirdLife International, 2013).

## **Conservation**

For capturing, transporting and selling birds within Indonesia and for importing and exporting them, the permits are required according to Indonesian legislation. For species that can be taken from the wild are established harvest quota and they can be taken just from specified places. The Black-and-white Laughingthrush is not listed as a protected species in Indonesia, but the harvest quota was established to zero for this species. Even though it is illegally sold openly in the bird markets (Shepherd, 2006; Shepherd, 2007). For conservation of this species the authorities should protect it by monitoring of the bird markets and the illegal trade and by enforcing the dealers to follow the laws. The public should be educated about the conservation needs of this species and more conservation institutions and the government in Indonesia should participate on its protection (Sepherd, 2006; Shepherd, 2007; Shepherd 2010).

To enhance and ensure the back-up population European zoos increased their holdings of Black-and-white Laughingthrush. In 2012 were recorded 37 individuals in EAZA institutions and few privately owned. The conservation breeding program was also created in Cikananga Wildlife Center and the species is successfully bred in this establishment since 2011 (Collar et al., 2012).

## **2.2 Monitoring of endangered species**

There is a great need for avian monitoring in conservation efforts (Brandes, 2008). Monitoring of endangered species can provide the information about the status of the species and can be used as a control tool whether the conservation efforts are successful. These information can give a clue which conservation steps and decisions should be made for the further protection of the species (Nichols and Williams, 2006; Morris et al., 2002). Birds are very good indicators of the environmental conditions, mainly of unexpected changes, because these changes have influence on the population size of many species (Koskimies, 1989).

### **Non-invasive acoustic monitoring**

Acoustic monitoring may be used in various ecosystems and many relatively easy methods may be provided to study acoustic signals of various species as recording, analysing, synthesising and using playback. All of these actions are relatively available

with small economic budget and sensitive in many aspects of impact and may provide valuable conservation effects (Laiolo, 2009). Acoustic monitoring brings new findings about the species as the information about the communication within the species, seasonal variability in acoustic behaviour and its relation with the ecological factors, and the variability of the animal's acoustic habitats (Blumstein, 2011). This method assumes that the acoustic communication in birds have some individually distinctive signature. According to Terry et al. (2005) vocal individuality need to fulfill several requirements that it can be used as an effective conservation tool such as easily recordable vocalization, simple size similar to number of individuals for discrimination analysis and consideration in choosing parameters to measure. Vocal individuality thus can be used to monitor specific individuals over time for obtaining more detailed information about the species as territorial and mating behaviour, population dynamics and habitat use (Terry et al., 2005; Mennill, 2011; Xia et al. 2012).

The acoustic monitoring provides many advantages as long-term recording without the observer. It gives the possibility of recording at larger scale of bird species, including nocturnal species and species with lower acoustic activity (Bardeli et al., 2010; Odom et al., 2013). The main advantage of individual acoustic monitoring is that it is a non-invasive technique (Terry et al., 2005). It means that it is very suitable for monitoring of species in environments where the animals are difficult to observe. It is also a very good tool for monitoring the species which are very sensitive to human approach, because capture-mark individual tracking techniques can have a negative long-term impact on behaviour of the individual (Grava et al., 2008; Fernández-Juricic et al., 2009; Mennill, 2011; Xia et al., 2012; Linhart et al., 2014). In this method it is necessary to develop suitable recognition system which will provide significant result in every acoustic environment (Bardeli et al., 2010). For the reliability of this recognition system of the acoustic monitoring is important the individuality of sound signals of the studied species (Grava et al., 2008). The recognition techniques need to provide stable information as easily recordable vocalizations and steady individual differences over time within the social context and body conditions of the species (Xia et al., 2012, Klenova et al., 2009).

The acoustic monitoring brings also disadvantages, for example when working with unsupervised recordings performed in an acoustically unpredictable area, where a great amount of overlap between different bird vocalizations and other noise sources may occur (Bardeli et al., 2010). In some species this method may not be able to be used, because if

the vocalization will change between years, it would not be possible to determine whether the individual and its vocalization is different (Terry et al., 2005).

### **2.3 Acoustic Communication**

Acoustic communication is very important in the life of birds in many aspects (Kumar, 2003). Birds use acoustic signals as messages to mate interactions and territory determinations and these messages carry information about the individuals as sex, mate and parental suitability, and individual identity (Westcott, 2001; Wanker and Fischer, 2001). Many types of vocalizations are used and each has specific biological function. These acoustic signals vary from long and complex (songs) to short and simple (calls) and appear in different types of context. They are characterized by the frequency, duration and amplitude (sound spectrogram technique) and the acoustic repertoire of the species typically differs in structure and the situation of use (Kumar and Bhatt, 2000; Kumar, 2003; Gammon and Baker, 2004). The size of the repertoire and its determination and comparison within the species is very important for the studies of behavioural ecology and the vocal signals can be used for estimation of the population size (Garamszegi et al., 2001; Kumar, 2003).

Acoustic signals are effectively used as a transmitter of information (Kumar, 2003). Bird song is used as a tool for managing the behaviour of another individual (Stutchbury and Morton, 2001). According to Reers et al. (2011) this behavior management is performed by signalling system which includes three parts: the sender, the signal and the receiver (Fig.4). Sender transmits the vocal signal that carries the acoustic information to receiver who decodes that information (Searby et al., 2004). The receivers then decide by the respond to the signal about the outcome of the communication (Stutchbury and Morton, 2001). Vocal signals contain different types of information depending on various contexts (e.g. territoriality, mating, and parental care) (Mouterde et al., 2014). The crucial factor in acoustic communication is therefore what signals mean to the senders and receivers. It is suggested that the bird song reflect the state of the sender and its following behaviour within the other contexts of the communication as surround conditions (Gill and Bierema, 2013).

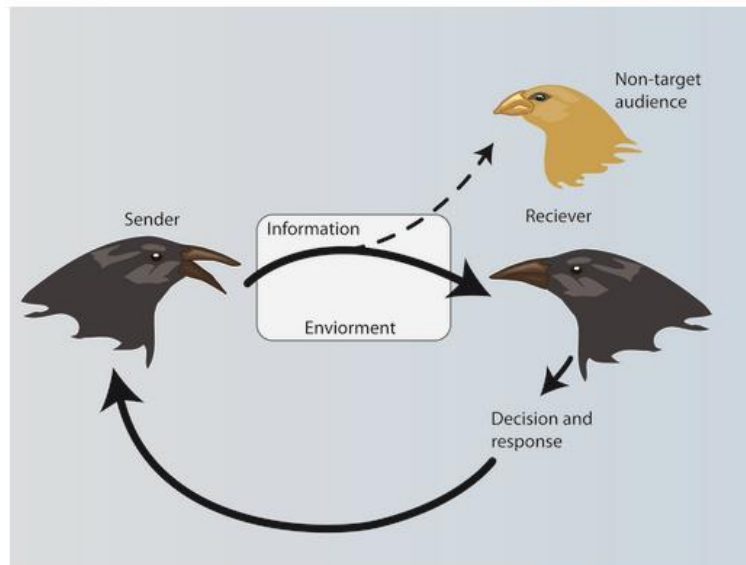


Fig. 4. Signalling system in birds including sender, signal and receiver (Gillam, 2011).

## 2.4 Individual recognition

As Dale et al. (2001) present, the fact that birds can identify specific individuals has been observed in many species. For social animals is beneficial to recognize their conspecifics or to be able to determine whether the individual is known by repeated contact (Stoddard et al., 1991). According to Tibbetts and Dale (2007) some of these benefits include decreased intrusion by neighbours in territorial interactions, decreased aggression between individuals of the same population and increasing stability in the population. Many avian species are able to recognize their conspecifics as their mates or offspring, and they can differentiate if the individual is the related one or if it belongs to different social class (Wanker and Fisher, 2001). The vocalization as the key for individual recognition has been observed in many bird species (Vignal et al., 2007; Xia et al., 2012). Vocal individuality and the process of vocal learning and obtaining the characteristic individual features may be influenced by a series of genetic, developmental, social and environmental conditions (Terry et al., 2005; Klenova et al., 2011). Individual recognition occurs when an individual can identify these characteristic features of another organism. The level of accuracy of individual recognition depends on the recognition cues of each individual. The more unique cues the individual has, the better will be that individual recognized (Dale et al., 2001).

Information about the individuality (acoustic fingerprint) is very important in vocal communication and both signallers and receivers benefit if the receivers can recognize the specific individually characteristic cues in the signals (Mouterde et al. 2014; Dabelsteen and Couchoux, 2015). For the individual recognition is important that the receivers learn and memorize specific distinctive characteristics of the signallers that are constant over time and in the future interaction they can recognize and discriminate the senders according to these characteristics (Lengagne et al., 1997; Wanker and Fischer, 2001; Terry et al., 2005; Tibbetts and Dale, 2007). For the recognition of individuals is required that the signaller provides inter-individual variability much higher than intra-individual variability, to be able to be recognized by the receiver. The signals has to be stereotyped within individual, but have to be different among individuals of the population (Lengagne et al., 1997; Wanker and Fischer, 2001; Lovell and Lein, 2005; Terry et al., 2005; Vignal et al., 2007; Reers et al., 2011).

Individual recognition is essential for a wide range of social interactions and for establishing the social systems (Wanker et al., 1998; Tibbetts and Dale, 2007). The individual vocal distinctiveness is very important in communication between male and female (especially in monogamous species). It is essential in keeping the pair bond between mates, in the recognition of known individuals or strangers for territorial species, in the parent-offspring relationship where the individual recognition can increase the success of breeding, and in the nest mate relationship (Wanker et al., 1998; Tibbetts and Dale, 2007; Mouterde et al. 2014). The identification of the individual cues in these contexts is beneficial, because it might decrease aggression, increase breeding success and it can positively influent the detection of predator (Wascher et al., 2012).

## **2.5 Vocal Repertoire**

The variability in singing behavior, the vocal repertoire, was observed in many oscines within and among populations (Kroodsma and Canady, 1985, Beecher et al., 2000). The number of male's vocalization types determine the repertoire size and it ranges from one to more than 2,000 types in various species (Molles and Vehrencamp, 1999; Xia et al., 2013). Vocal repertoire size generally arises due to sexual selection in male-male competition and female choice and it can vary between males within one population (Kroodsma and Canady, 1985; Doutrelant et al., 2000; Stutchbury and Morton, 2001; Reid

et al. 2004). Given this fact, males with larger acoustic repertoire are more successful in many aspects as better physical condition, better survival rate, better territories and higher reproductive success (Searcy et al., 1985; Doutrelant et al., 2000; Hesler et al., 2012). This is particularly true for birds of temperate zone, for tropical species with year-long territories may not be so huge pressure of sexual selection on repertoire size (Stutchbury and Morton, 2001).

The functions of different types of vocal repertoire is the same, they are used to defend the territory and to attract the mate, and these call types are interchangeable. The reason for using different types of vocalization is to provide higher song diversity (Beecher et al., 1996; Beecher et al., 2000; Xia et al., 2013). Diversity varies between species, in some species just male provide the acoustic repertoire, in others the females participate on the vocal structure of the species by individual vocalization or in duetting with males (Kumar, 2003).

### **Duet**

According to Rogers et al. (2006) duets are defined as accurate and temporal coordination (often stereotyped) of vocalization by two individuals at the same time, especially by mated pair and it has been observed in many tropical birds (Rogers et al., 2002; Kumar, 2003; Logue et al., 2008). Duets vary in many aspects as degree of synchronization, complexity and duration, and also in aspects of each duetting individuals as the sex of the initiator of duetting, differences in male and female part of the duet and frequency of expressing duet in the comparison with solo vocalization (Rogers et al., 2002).

Duets may carry information about the identity of pair and about their territory range, or about the distance between individuals of the pair (Logue, 2007). The main function of duet is primarily territory defense. It includes joint territory defence and share resources, prevention and secure of the mate desertion, and prevention from being usurped from same-sex rivals (Seddon and Tobias, 2006; Rogers et al., 2007; Logue et al., 2008).



### **3 Aims of the thesis**

The thesis had the following aims:

- Basic description of voice repertoire of Black-and-white Laughingthrush in the nesting season
- Test of individual differences among different types of signals
- Determine which specific acoustic parameters encode individual identity.
- Rate of individual differences depending on sex, social type and context.

The hypotheses of the research were:

- 1) According to the behavioural ecology of the Black-and-white Laughingthrush (territoriality and hidden way of life), it is expected that individuals will recognize each other by the voice.
- 2) I assumed that some calls from the repertoire would be individually specific.

## 4 Material and Methods

### 4.1 Study Site

The research took place in Cikananga Wildlife Rescue Center in West Java, Indonesia. It is located in the hilly area of the southern of Sukabumi (Cikananga Wildlife Center, 2015) (see Fig.5). This area is located in the mountain tropical and subtropical moist broadleaf forest, with the average rainfall from 3 000 to 5 000 mm per annum (WWF, 2015; Tempat wisata pulau Jawa, 2015). The Java Indonesia climate has two seasons, the wet season from October to April and dry season from May to September. The relative humidity reached from 79 to 82%. The average temperature is from 22 to 29°C (Tempat wisata pulau Jawa, 2015).

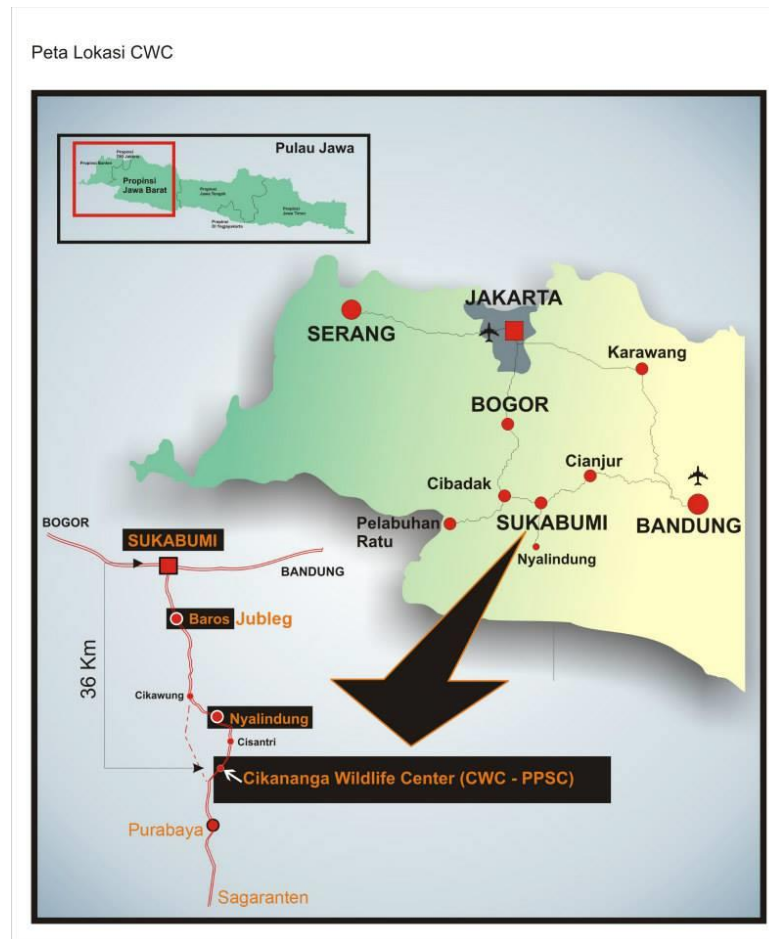


Fig. 5. The location of Cikananga Wildlife Rescue Center in West Java, Indonesia (Cikananga Wildlife Center, 2015)

Cikananga Wildlife Rescue Center is a non-profit organization dedicated to the conservation of wildlife and its habitat in Indonesia. It covers an area of 14 hectares and it has 3 750 animals rescued. It belongs into the largest wildlife rescue centers in Indonesia. It was founded between the years 2000 and 2002 as one of the eight Wildlife Rescue Centers in Indonesia as the implementation of CITES. It was established to stimulate and assist law enforcement of illegal protected wildlife trade and to provide the placement for confiscated animals. Beside this main aim of the center it has other activities as education, rescuing animals and conservation breeding programs (Cikananga Wildlife Center, 2015).

## **4.2 Study Birds**

Black-and-white Laughingthrush is bred at the center in conservation breeding program to create a sustainable captive population. The breeding program of this species was established in 2008 by 13 wild birds rescued from the bird market. According to the bad condition of the birds, just 10 birds survived, seven females and three males and from those three pairs were formed (Cikananga Wildlife Center, 2015). According to the studbook of Cikananga Wildlife Center at the date of 22nd of February 2013, 20 individuals in total were bred, 9 females and 11 males. During the breeding program, including the three individuals in 2008, 13 individuals died.

At the time of my research 35 individuals of Black-and-white Laughingthrush in total (without the newly hatched chicks) were bred: 13 females, 17 males and 5 individuals of unknown sex. The birds were divided into 11 pairs, one male and one female were in separate enclosures, three males were in one enclosure, one female with two males were in one enclosure and three individuals of unknown sex were together in one enclosure. The birds were kept in five different buildings: Block A, Block B, R5, PKBI 1 and PKBI 2. Each building housed at least one or several pairs and separate individuals.

## **4.3 Recording**

I recorded 30 individuals of Black-and-white Laughingthrush from November to December 2013. The records were made during the dusk at 5:30 a.m. to 9:30 a.m. because at different times they have shown no or little vocalisation. For record I used Olympus LS-5 linear PCM recorder with field microphone Sennheiser ME66. I made two recording sessions.

The 1st session was recorded from 22nd of November to 5th of December. In total 28 individuals were recorded: eight pairs, nine mature individuals and three immature of unknown sex. The individuals were kept in 14 enclosures and I recorded 15 minutes for one enclosure each day. The following table (Tab.1) presents the placement of the enclosures and its inhabitants (in the five buildings) for the session one.

Tab. 1. Recording Cycle 1: Number of individuals in each building and number of enclosures, individuals, pairs and separate individuals recorded.

Building	N Enclosures Housed	N Enclosures Recorded	N Individuals Housed	N Individuals Recorded	N Pairs Recorded	Other individuals recorded (Female – F, Male – M) (separate/separate/...)
Block A	3	3	6	5	1	1F/2M
Block B	2	2	4	4	2	-
R5	2	2	5	5	1	1F2M
PKBI 1	3	-	6	-	-	-
PKBI 2	8	8	14	14	4	1M/1M/1F/3?
<b>Total</b>	<b>18</b>	<b>15</b>	<b>35</b>	<b>28</b>	<b>8</b>	<b>12</b>

After the recording in the 1st session new pairs were formed and some individuals were moved to another enclosures and I was allowed to record in new enclosures. According to that the 2nd session was designed as follows.

The 2nd session was recorded from 8th to 16th of December. 18 individuals of nine pairs were recorded in total. In this session I recorded just pairs, because they showed most vocalization in previous recording cycle. The pairs were kept in nine enclosures and I recorded 25 minutes for one enclosure each day. The following table (Tab.2) present the placement of the enclosure and its inhabitants (in the five buildings) for the session two.

Tab. 2. Recording Session 2: Number of individuals in each building and number of enclosures, individuals, pairs and separate individuals recorded.

Building	N Enclosures Housed	N Enclosures Recorded	N Individuals Housed	N Individuals Recorded	N Pairs Recorded	Other individuals recorded
Block A	3	1	6	2	1	-
Block B	2	2	4	4	2	-
R5	2	-	5	-	-	-
PKBI 1	3	2	8	4	2	-
PKBI 2	8	4	12	8	4	-
<b>Total</b>	<b>18</b>	<b>9</b>	<b>35</b>	<b>18</b>	<b>9</b>	<b>-</b>

For the more precise information of the vocalization I also wrote down the information about the surround as context which were: answer – when the individuals react to each other, approach – when the keeper or me disturbed the individuals, cat – when the cat was nearby the enclosures, observing – when the individuals were hopping around the enclosure, bonding – when the individuals were interact between themselves, standard – when I did not find any specific cause of the vocalization.

#### 4.4 Sampling

I recorded each day from 5:30 to 9:30 a.m. I recorded pairs or individuals in separate enclosure or two individuals in neighboring enclosures. Each enclosure got number, in first session 1 to 14, in second session 1 to 9. In first session I recorded one enclosure for 15 minutes, in the second session 25 minutes. Recording order was in systematic design, first day I started recording with enclosure number one, second day I started with enclosure number two, third day I started with enclosure number three and so on. The recording sessions and the marking of the enclosures and its inhabitants are described in the following tables (Tab.3, Tab.4).

Tab. 3. Recording Session 1: Marked enclosure from 1 to 14, with their inhabitants and its social type (pair - 1+1, male alone - 1+0, female alone - 0+1, two males together - 2+0, two males and one female - 2+1, unknown sex - ?)

Enclosure	N Individuals	Social Type
1	2	1+1
2	2	1+1
3	2	1+1
4	3	2+1
5	2	1+1
6	2	1+1
7	2	1+0, 1+0
8	1	0+1
9	2	1+1
10	2	1+1
11	3	?
12	2	1+1
13	2	2+0
14	1	0+1

Tab. 4. Recording Session 2: Marked enclosure from 1 to 9, with their inhabitants and its social type (pair - 1+1, pair with hatched chicks - 1+1+)

Enclosure	N Individuals	Social Type
1	2	1+1
2	2	1+1
3	2	1+1
4	2	1+1
5	2	1+1
6	2	1+1
7	2	1+1
8	2	1+1
9	2	1+1+

#### 4.5 Acoustic analysis

For the inspection of the quality of calls I used Avisoft-SASLab Demo version. The spectrogram parameters were used with the following parameters: Hamming window, FFT-length 256 points, frame 50%, overlap 0%. According to those settings the bandwidth reached 244 Hz, the frequency resolution was 188 Hz and the time resolution 5,3 ms. For further analysis I chose the records with good visible quality of the spectrogram and with no significantly distorting noise, which destroyed the recorded vocalization of Black-and-white Laughingthrush. Each song were sampled at 48 kHz and stored as 16-bit wav. format files.

For the analysis I used software RavenPro v. 1.4. with following spectrogram parameters: Blackmann window type, size 520 samples, overlap 85%. These settings provided bandwidth 151 Hz, DFT size 1024 samples and grid spacing 46,9 Hz. As measurements I chose the parameters, described in the following table (Tab.5). These parameters were measured from automatic procedures of the software. In addition to these parameters I manually measured contact song in males, trill and contact song in females and duet in both sex (see Tab.6). These parameters mainly represent the details in the structure of calling of both individuals (Fig.6).

Tab. 5. Description of measured parameters chosen for the acoustic analysis of Black-and-white Laughingthrush in the RavenPro v. 1.4 (Charif et al., 2010).

Parameter	Description
<b>Aggregate Entropy</b>	The aggregate entropy measures the disorder in a sound by analysing the energy distribution within a selection. Higher entropy values correspond to greater disorder in the sound whereas a pure tone would have zero entropy.
<b>1st Quartile Frequency (Hz)</b>	The frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the selection. The summed energy has to exceed 25% of the total energy.
<b>3rd Quartile Frequency (Hz)</b>	The frequency that divides the selection into two frequency intervals containing 75% and 25% of the energy in the selection. The summed energy has to exceed 75% of the total energy.
<b>IQR (Inter-quartile Range) Bandwidth (Hz)</b>	The difference between the 1st and 3rd Quartile Frequencies.
<b>Frequency 5% (Hz)</b>	The frequency that divides the selection into two frequency intervals containing 5% and 95% of the energy in the selection. The summed energy has to exceed 5% of the total energy.
<b>Frequency 95% (Hz)</b>	The frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy in the selection. The summed energy has to exceed 95% of the total energy.
<b>Bandwidth 90% (Hz)</b>	The difference between the 5% and 95% frequencies.
<b>1st Quartile Time (s)</b>	The point in time that divides the selection into two time intervals containing 25% and 75% of the energy in the selection. The summed energy has to exceed 25% of the total energy.
<b>3rd Quartile Time (s)</b>	The point in time that divides the selection into two time intervals containing 75% and 25% of the energy in the selection. The summed energy has to exceed 75% of the total energy.
<b>IQR (Inter-quartile Range) Duration (s)</b>	The difference between the 1st and 3rd Quartile Times.
<b>Time 5% (s)</b>	The point in time that divides the selection into two time intervals containing 5% and 95% of the energy in the selection. The summed energy has to exceed 5% of the total energy.
<b>Time 95% (s)</b>	The point in time that divides the selection into two time intervals containing 95% and 5% of the energy in the selection. The summed energy has to exceed 95% of the total energy.
<b>Duration 90% (s)</b>	The difference between the 5% and 95% times.
<b>RMS Amplitude</b>	The root-mean-square amplitude (sometimes called "effective amplitude") of the selected part of the signal.
<b>Max Time (s,%)</b>	The first time in the selection at which a sample with amplitude equal to Max Amplitude occurs.
<b>Min Time (s,%)</b>	The first time in the selection at which a sample with amplitude equal to Min Amplitude occurs.
<b>Peak Time (s)</b>	The first time in the selection at which a sample with amplitude equal to Peak Amplitude occurs.

Tab. 6. Description of manually measured parameters chosen for the acoustic analysis of Black-and-white Laughingthrush.

Parameter	Description	Call Type	Sex (F-female, M-male)
<b>Duration of the vocalization (s)</b>	The total duration of the expressed vocalization.	duet, trill, twitter, contact song	F,M
<b>Cadency</b>	Cadency of the male and female part of vocalization within the duet.	duet	F,M
<b>Male %</b>	Duration of male vocalization in duet.	duet	M
<b>Duration Female (s)</b>	Duration of female vocalization in duet.	duet	F
<b>Duration M1 (s)</b>	Duration of male vocalization in duet.	duet	M
<b>Duration M2 (s)</b>	Duration of the second part of male vocalization in duet if the male expressed it.	duet	M
<b>Duration of twitter part M (s)</b>	Duration of male twitter vocalization in duet.	duet	M
<b>Interval M1-M2 (s)</b>	Duration of the interval between the male vocalization in duet.	duet	M
<b>Start Sex</b>	The sex of individual who started duet.	duet	F,M
<b>Start Time (s)</b>	Start time of male vocalization according to female in duet.	duet	F,M
<b>M elements</b>	Number of the elements of male vocalization in duet.	duet, contact song	M
<b>Trill Cadency</b>	Number of trill elements in trill vocalization.	trill	F,M
<b>F Trill Cadency</b>	Number of trill elements of female vocalization in duet and contact song (measured for 0,5 s from the whole vocalization).	duet, contact song	F
<b>High Frequency (Hz)</b>	Manually measured the highest frequency of the determined duet.	duet	F,M
<b>Low Frequency (Hz)</b>	Manually measured the lowest frequency of the determined duet.	duet	F,M
<b>Frequency Range (Hz)</b>	The difference between the highest and lowest frequency.	duet	F,M



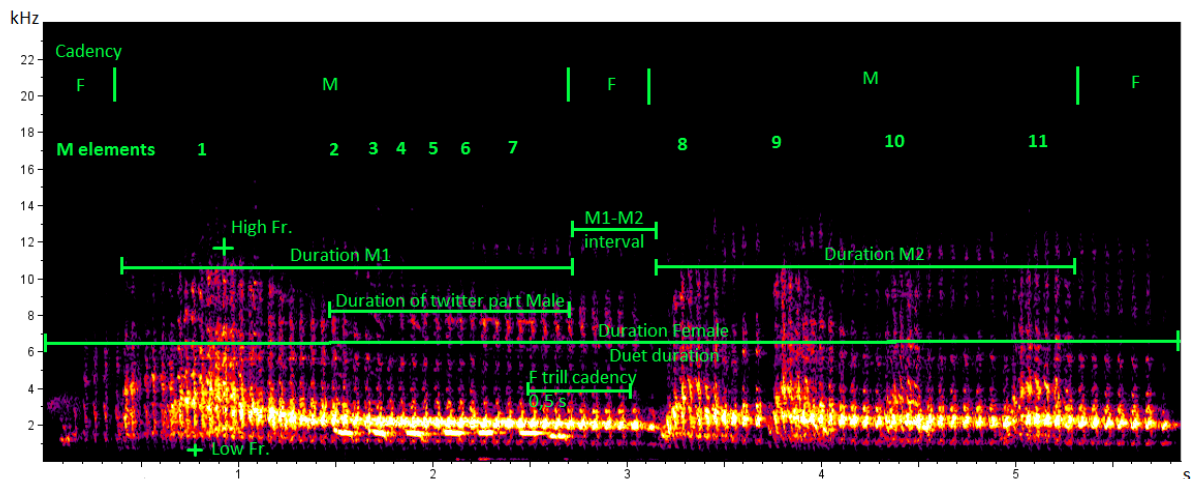


Fig. 6. The manually measured parameters in spectrogram of duet: Cadency, Duration of the duet, Duration Female, Duration M1, Duration M2, Interval M1-M2, Duration of twitter part of male, F trill cadency, High Frequency and Low Frequency. In this specific duet the duration of the duet was equal to duration of the female call, the cadency was FMFMF and the number of the elements of male part was 11.

#### 4.6 Statistical Analysis

For each individual I used one to ten signal samples to analyse each type of the vocalization, in total I analysed 117 samples of twitter, 131 samples of trill, 63 samples of contact song and 95 samples of duet. I used descriptive statistics for the measured parameters of each call types where I monitored the minimum and maximum values and its average with the standard deviation: min-max (mean  $\pm$  SD).

The influence of social type, context and sex on the expressed calls was tested by parametric tests. To analyse impact of a single categorical independent variable on the repertoire in situations where the other categories contained small sample sizes I used one-way univariate analysis of variance (ANOVA). To analyse the interactive effects of two categories and its influence on the manifested calls I used factorial analysis of variance. To determine the differences between each variable of category Scheffe post-hoc tests were computed.

Nonparametric Mann-Whitney U Test was used for the comparison of sex within the measured parameters of each call type. I used the nonparametric test because some categories included smaller sample of data set.

The individuality was analysed in different aspects. At first I used univariate analysis of variance – one-way ANOVA on the parameters of each type of vocalization to determine in which is the significant difference among individuals. Second I described the intra-individual and inter-individual variations of each parameter by using the coefficient of variation (CV) according to Bloomfield et al. (2005). The mean coefficient of variation within the individuals (mean  $CV_W$ ) was calculated for each individual. The coefficient of variation among individuals ( $CV_A$ ) was also calculated using the descriptive statistics for each parameter of the expressed calls for all individuals. Then I calculated the potential for individual coding (PIC) by dividing the  $CV_A$  by the mean  $CV_W$  values across all individuals for the parameters of each type of vocalization. When the PIC value is greater than one in the specific parameter, this parameter can be used for the individual recognition because its variability among the different birds is greater than the variability among the individual bird (Laiolo et al., 2000; Aubin et al. 2004; Vignal et al., 2007).

To test the differences between the intra- and inter-individual variability among the call types I used the nonparametric Friedman test.

The discriminant function analysis (DFA) was conducted, to examine whether the pairs could be correctly classified by its duet parameters. The stepwise discriminant analysis with one-out cross-validation procedure was computed on the 32 duet parameters to determine which parameters were the most important in distinguishing individuals (Fitzsimmons et al., 2008; Ręk and Osiejuk, 2011; Fernández et al., 2012). The cross validation is a type of measuring the predictive performance of a statistical model. It splits the data set on training sample for examining the parameters of data set, and validation sample for estimating the risk of these parameters. The cross validation then select the parameters with the smallest estimated risk. In leave-one-out cross validation is each data set parameter left out and used for the validation (Sylvain and Celisse, 2010). The percentage of correct matching and its cross-validated values indicate the reliability of distinguishing vocalizations based on the duet parameters used in the analysis (Davis, 1986). Because the sample size of twitter, trill and song was too small DFA was used only for determining pairs by duet.

Statistical analyses were carried out in the programme Statistica 12 and in software IBM SPSS Statistics 19. All p-values were extracted from two tailed statistical tests. Because I made multiple statistical tests on the same data set I used the Bonferroni correction to reduce the errors by false-positive results.

## 5 Results

I have recorded 990 vocalizations of 24 individuals of Black-and-white Laughingthrush totally. The rest 11 individuals (six mature individuals not formed into pairs, three individuals of unknown sex and two newly hatched chicks) were excluded from the analysis due to difficulties with their marking during the recording.

I recorded four different types of vocalization: twitter, trill, contact song and duet. The following table (Tab.7) summarizes the various vocalizations and their total number and number of individuals who have expressed it.

Tab. 7. The summary of call types of Black-and-white Laughingthrush: number of each call performed, number of individuals who expressed it and number of males and females who expressed it, and total number of recorded individuals, recorded males, recorded females and total number of recorded vocalizations.

Call type	N individuals	Male	Female	N calls	Percentage
Duet	22	11	11	242	49
Trill	22	12	10	193	19,5
Twitter	22	12	10	170	17
Contact song	21	12	9	143	14,5
Total	24	13	11	<b>990</b>	100

### 5.1 Repertoire

#### Twitter

The twitter occurred in both sex almost equally. The call was expressed in ordinary moments during the whole recording, when the individuals were hopping over the enclosure and were not disturbed. The following table (Tab.8) describes the representation of the twitter according to sex and context. The duration of twitter reached 0,04 – 1,14 s ( $0,14 \pm 0,11$ ) (see Tab.9). For the spectrogram of twitter see Fig.7. The description of all measured acoustic parameters of trill used in the statistic is in Tab.10 and the correlation of these parameters is represented in Tab.11.

Tab. 8. The description of the twitter: representation of sex and context.

TWITTER	Sex		Context					
	Male	Female	Answer	Approach	Cat	Standard	Bonding	Observing
Number of Vocalization	90	80	1	6	1	145	0	17
Percentage	52,9	47,1	0,6	3,5	0,6	85,3	0	10

Tab. 9. Descriptive statistic of the twitter: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.

Variable	Mean	Minimum	Maximum	Std.Dev.
Duration Male	0,15	0,04	0,48	0,07
Duration Female	0,14	0,04	1,14	0,15

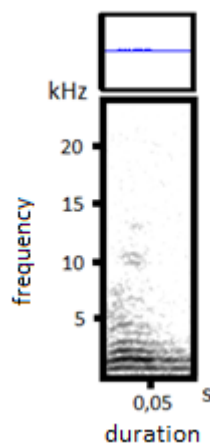


Fig. 7. Spectrogram and waveform of twitter.

Tab. 10. Description of measured acoustic parameters of twitter used for the statistical analysis.

<b>Twitter parameters</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Std.Dev.</b>
Duration (s)	0,142	0,041	1,142	0,112
Q1 Freq (Hz)	1436,697	515,600	2390,600	315,611
Q1 Time (s)	33,244	0,900	106,500	19,626
Q3 Freq (Hz)	1949,521	1312,500	5015,600	834,571
Q3 Time (s)	33,274	0,900	106,500	19,630
Agg Entropy (u)	4,097	2,664	6,689	0,941
BW 90% (Hz)	1365,385	234,400	5203,100	1154,776
Dur 90% (s)	0,091	0,000	1,000	0,101
Freq 5% (Hz)	1062,100	140,600	1640,600	329,465
Freq 95% (Hz)	2427,485	1359,400	6375,000	1196,820
IQR BW (Hz)	512,821	93,800	3234,400	699,156
IQR Dur (s)	0,014	0,000	0,500	0,054
Time 5% (s)	33,225	0,900	106,500	19,620
Time 95% (s)	33,307	0,900	106,500	19,634
Max Time (s)	33,252	0,883	106,480	19,625
Max Time %	36,921	13,063	84,127	14,656
Min Time (s)	33,251	0,883	106,472	19,625
Min Time %	36,747	9,009	82,812	14,148
Peak Time (s)	33,250	0,883	106,480	19,624
RMS Amp (u)	316,036	68,600	3385,300	437,840

Tab. 11. Correlations of measured parameters of twitter, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.

	Duration Twitter	<b>1,00</b>	0,11	0,20	0,18	0,89	0,30	0,25	0,10	0,85	0,10	0,11	0,14	0,11	0,11	0,15	0,11	0,17
	Q1 Freq (Hz)	0,34	<b>1,00</b>	0,23	0,36	0,17	0,67	0,53	0,25	0,13	0,25	-0,15	-0,33	-0,15	-0,15	-0,15	-0,15	0,17
	Q1 Time (s)	0,11	-0,15	<b>1,00</b>	-0,10	0,16	-0,11	-0,12	-0,04	0,13	-0,04	<b>1,00</b>	0,12	<b>1,00</b>	0,02	<b>1,00</b>	0,02	-0,10
	Q3 Freq (Hz)	0,21	0,58	-0,09	<b>1,00</b>	0,16	0,36	<b>0,91</b>	<b>0,93</b>	0,14	<b>0,93</b>	-0,09	-0,28	-0,09	-0,26	-0,09	-0,09	-0,08
	Q3 Time (s)	0,11	-0,15	<b>1,00</b>	-0,10	0,16	-0,11	-0,12	-0,04	0,14	-0,04	<b>1,00</b>	0,12	<b>1,00</b>	0,02	<b>1,00</b>	0,02	-0,10
	Agg Entropy (u)	0,20	0,23	0,00	<b>1,00</b>	0,27	-0,06	0,87	0,80	0,23	0,80	0,01	0,00	-0,17	0,00	-0,20	0,00	-0,22
	BW 90% (Hz)	0,18	0,36	-0,10	<b>1,00</b>	0,22	0,00	<b>0,96</b>	0,84	0,20	0,84	-0,10	-0,10	-0,23	-0,10	-0,22	-0,10	-0,23
	Dur 90% (s)	0,89	0,17	0,16	0,22	<b>1,00</b>	0,07	0,23	0,12	0,83	0,12	0,16	0,16	0,16	0,16	0,14	0,16	-0,02
	Freq 5% (Hz)	0,30	0,67	-0,11	0,36	-0,11	<b>1,00</b>	0,27	0,13	0,10	0,13	-0,11	-0,29	-0,11	-0,22	-0,11	-0,22	0,36
	Freq 95% (Hz)	0,25	0,53	-0,12	<b>0,91</b>	-0,12	0,27	<b>1,00</b>	0,85	0,22	-0,12	-0,12	-0,30	-0,12	-0,27	-0,12	-0,12	-0,13
	IQR BW (Hz)	0,10	0,25	-0,04	<b>0,93</b>	-0,04	0,13	0,85	<b>1,00</b>	0,10	-0,04	-0,04	-0,19	-0,04	-0,20	-0,04	-0,17	-0,17
	IQR Dur (s)	0,85	0,13	0,13	0,14	0,14	0,23	0,20	0,10	<b>1,00</b>	0,13	0,14	0,13	0,00	0,13	-0,04	0,13	0,03
	Time 5% (s)	0,10	-0,15	<b>1,00</b>	-0,09	0,16	-0,11	-0,12	-0,04	0,13	-0,04	<b>1,00</b>	0,12	<b>1,00</b>	0,02	<b>1,00</b>	0,02	-0,10
	Time 95% (s)	0,11	0,34	-0,09	0,14	0,16	-0,11	-0,12	-0,04	0,13	-0,04	<b>1,00</b>	0,12	<b>1,00</b>	0,02	<b>1,00</b>	0,02	-0,10
	Max Time %	-0,14	-0,33	0,12	-0,23	-0,10	-0,29	-0,30	-0,19	-0,04	-0,19	-0,04	-0,20	-0,04	-0,20	-0,04	-0,17	-0,10
	Min Time %	0,11	-0,15	<b>1,00</b>	-0,10	0,16	-0,11	-0,12	-0,04	0,13	-0,04	<b>1,00</b>	0,12	<b>1,00</b>	0,02	<b>1,00</b>	0,02	-0,10
	Peak Time %	-0,19	-0,26	0,02	-0,20	-0,14	-0,22	-0,27	-0,20	-0,04	-0,20	0,02	0,63	0,03	<b>1,00</b>	0,03	0,03	-0,10
	RMS Amp (u)	0,17	0,17	-0,10	-0,23	-0,02	0,36	-0,13	-0,17	0,03	-0,17	-0,10	-0,10	-0,10	-0,10	-0,10	-0,10	<b>1,00</b>

## Trill

The trill occurred in both sex, male and female. Females expressed it twice time often than males. It was expressed during the whole recording and it was mainly a sign of some small distraction like vocalization of other bird species or small fright from approach of the keeper or me, but did not result in greater distress. The following table (Tab.12) describes the representation of the trill according to sex and context. Male duration of trill reached 0,04 - 1,82 s ( $0,36 \pm 0,28$ ; mean  $\pm$  standard deviation), female duration of trill reached 0,05 - 1,12 s ( $0,28 \pm 0,18$ ) (see Tab.13). For the spectrogram of trill see Fig.8. The description of all measured acoustic parameters of trill used in the statistic is in Tab.14 and the correlation of those parameters is represented in Tab.15.

Tab. 12. The description of the trill: representation of sex and context.

TRILL	Sex		Context					
	Male	Female	Answer	Approach	Cat	Standard	Bonding	Observing
<b>Number of Vocalization</b>	62	131	1	14	3	175	0	0
<b>Percentage</b>	32,1	67,9	0,5	7,2	1,6	90,7	0	0

Tab. 13. Descriptive statistic of the trill: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.

Variable	Mean	Minimum	Maximum	Std.Dev.
<b>Duration Male</b>	0,36	0,04	1,82	0,28
<b>Duration Female</b>	0,28	0,05	1,12	0,18

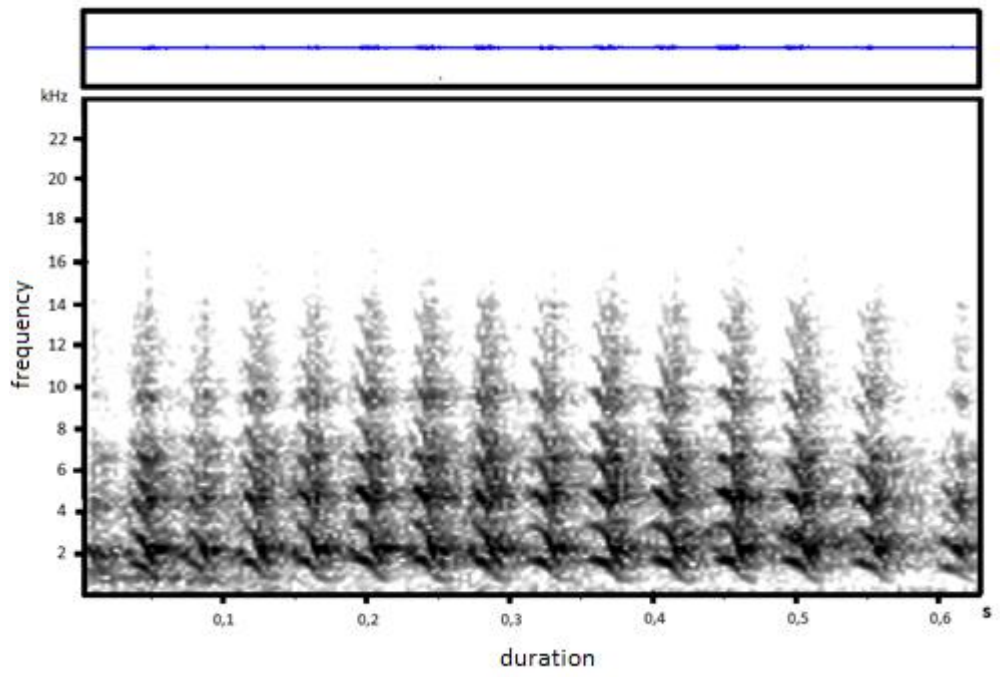


Fig. 8. Spectrogram and waveform of trill.



Tab. 14. Description of measured acoustic parameters of trill used for the statistical analysis.

<b>Trill parameters</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Std.Dev.</b>
Duration	0,307	0,040	1,820	0,228
Trill cadency	5,922	0,000	19,000	3,693
Q1 Freq (Hz)	1876,455	937,500	3609,400	474,388
Q1 Time (s)	32,557	0,300	94,000	20,311
Q3 Freq (Hz)	3302,326	1453,100	5859,400	1096,674
Q3 Time (s)	32,657	0,400	94,200	20,319
Agg Entropy (u)	5,832	2,774	6,822	0,719
BW 90% (Hz)	3521,438	234,400	6328,100	1299,831
Dur 90% (s)	0,240	0,000	1,200	0,186
Freq 5% (Hz)	1284,153	656,200	1921,900	241,903
Freq 95% (Hz)	4805,599	1875,000	7968,800	1390,222
IQR BW (Hz)	1425,871	93,800	3328,100	818,751
IQR Dur (s)	0,102	0,000	1,000	0,138
Time 5% (s)	32,531	0,300	94,000	20,310
Time 95% (s)	32,764	0,500	94,200	20,309
Max Time (s)	32,581	0,334	94,000	20,307
Max Time %	25,053	1,249	85,031	19,328
Min Time (s)	32,580	0,334	93,998	20,304
Min Time %	24,994	1,784	85,031	20,201
Peak Time (s)	32,583	0,334	94,000	20,302
RMS Amp (u)	290,184	70,800	3689,100	401,320

Tab. 15. Correlations of measured acoustic parameters of trill, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.

	<b>Duration</b>	1,00	0,66	0,14	-0,05	-0,05	0,06	-0,06	-0,09	0,96	0,17	-0,06	0,00	0,82	-0,05	-0,04	0,05	0,02	-0,05	0,23
	<b>F trill cadency alone</b>	0,66	1,00	0,17	-0,02	-0,02	0,23	0,23	0,12	0,68	0,11	0,14	0,17	0,56	-0,02	-0,02	-0,03	-0,07	-0,02	-0,08
	<b>Q1 Freq (Hz)</b>	0,14	0,17	1,00	-0,11	0,72	0,51	0,55	0,55	0,13	0,75	0,65	0,39	0,15	-0,11	-0,11	-0,06	-0,11	-0,11	0,21
	<b>Q1 Time (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,05	-0,06	1,00	0,06
	<b>Q3 Freq (Hz)</b>	0,06	0,20	0,72	-0,09	1,00	0,82	0,80	0,80	0,07	0,52	0,84	0,92	0,06	-0,09	-0,09	-0,07	-0,11	-0,09	0,11
	<b>Q3 Time (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,05	-0,07	1,00	0,06
	<b>Agg Entropy (u)</b>	-0,06	0,23	0,51	-0,11	0,82	-0,11	1,00	0,91	-0,02	0,22	0,89	0,80	-0,02	-0,11	-0,11	-0,17	-0,11	-0,11	-0,08
	<b>BW 90% (Hz)</b>	-0,09	0,12	0,55	-0,14	0,80	-0,14	0,91	1,00	-0,08	0,29	0,99	0,75	-0,10	-0,14	-0,15	-0,14	-0,18	-0,15	0,01
	<b>Dur 90% (s)</b>	0,96	0,68	0,13	-0,03	-0,08	-0,02	-0,08	1,00	0,15	1,00	-0,05	0,01	0,87	-0,03	-0,02	0,00	-0,02	-0,03	0,15
	<b>Freq 5% (Hz)</b>	0,17	0,11	0,75	0,13	0,52	0,22	0,29	0,29	0,15	1,00	0,45	0,27	0,11	-0,13	-0,13	0,07	-0,13	0,07	0,26
	<b>Freq 95% (Hz)</b>	0,17	0,11	0,75	0,13	0,52	0,22	0,29	0,29	0,15	1,00	0,45	0,27	0,11	-0,13	-0,13	0,07	-0,13	0,07	0,26
	<b>IQR BW (Hz)</b>	0,00	0,17	0,39	-0,06	0,92	0,80	0,75	0,75	0,01	0,27	0,75	1,00	-0,01	-0,06	-0,06	-0,06	-0,11	-0,06	0,03
	<b>IQR Dur (s)</b>	0,82	0,56	0,15	0,04	0,06	-0,02	-0,10	0,87	0,11	0,87	0,11	-0,07	-0,01	1,00	0,05	0,04	-0,01	0,04	0,07
	<b>Time 5% (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,05	-0,07	1,00	0,06
	<b>Time 95% (s)</b>	-0,04	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,05	-0,07	1,00	0,06
	<b>Max Time (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,04	-0,06	1,00	0,06
	<b>Max Time %</b>	0,05	-0,03	-0,06	-0,05	-0,07	-0,17	-0,14	0,00	0,07	0,07	-0,12	-0,06	-0,01	1,00	-0,05	1,00	0,80	-0,04	0,04
	<b>Min Time (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,04	-0,06	1,00	0,06
	<b>Min Time %</b>	0,02	-0,07	-0,06	-0,06	-0,11	-0,18	-0,15	-0,18	-0,02	0,07	-0,16	-0,11	-0,03	-0,07	-0,07	0,80	1,00	-0,06	0,08
	<b>Peak Time (s)</b>	-0,05	-0,02	-0,11	1,00	-0,09	-0,11	-0,14	-0,14	-0,03	-0,13	-0,16	-0,06	0,04	1,00	1,00	-0,04	-0,06	1,00	0,06
	<b>RMS Amp (u)</b>	0,23	0,08	0,21	0,06	0,11	0,06	0,07	0,01	0,15	0,26	0,03	0,03	0,07	0,07	0,04	0,07	0,04	0,07	1,00

## Contact song

The contact song occurred in both sex during the whole recording. This call is very similar to duet vocalization, but the individuals expressed it separately, without depending on the opposite sex. The conditions under which they expressed this vocalization were very similar to those exhibited in duet singing. But it occurred much more frequently as the interaction between the individuals. Males expressed it twice time often than females. The following table (Tab.16) describes the representation of the contact song according to sex and context. Male duration of contact song reached 0,74 – 10,13 (3,56 ± 2,2), female duration of contact song reached 0,34 – 5,97 s (1,03 ± 1,27) (see Tab.17). For the spectrogram of contact song for each sex see Fig.9 and 10. The description of all measured acoustic parameters of contact song used in the statistic is in Tab.18 and the correlation of those parameters is represented in Tab.19.

Tab. 16. The description of the contact song: representation of sex and context.

CONTACT SONG	Sex		Context					
	Male	Female	Answer	Approach	Cat	Standard	Bonding	Observing
Number of Vocalization	100	43	20	5	0	113	5	0
Percentage	69,9	30,1	14	3,5	0	79	3,5	0

Tab. 17. Descriptive statistic of the contact song: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded males and females.

Variable	Mean	Minimum	Maximum	Std.Dev.
Duration Male	3,56	0,74	10,13	2,20
Duration Female	1,03	0,34	5,97	1,27

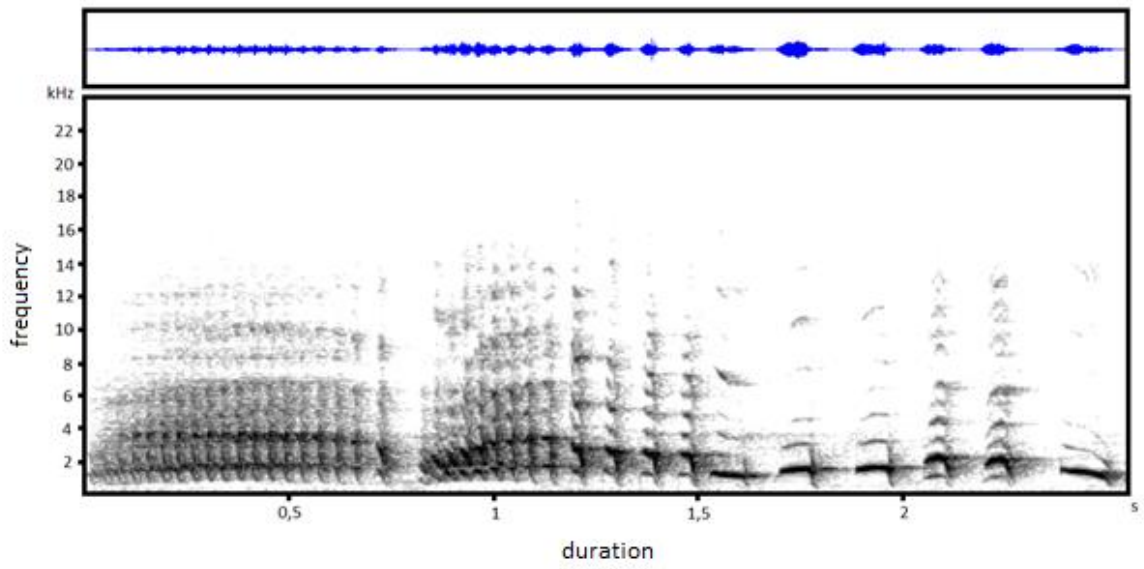


Fig. 9. Spectrogram and waveform of contact song - male.

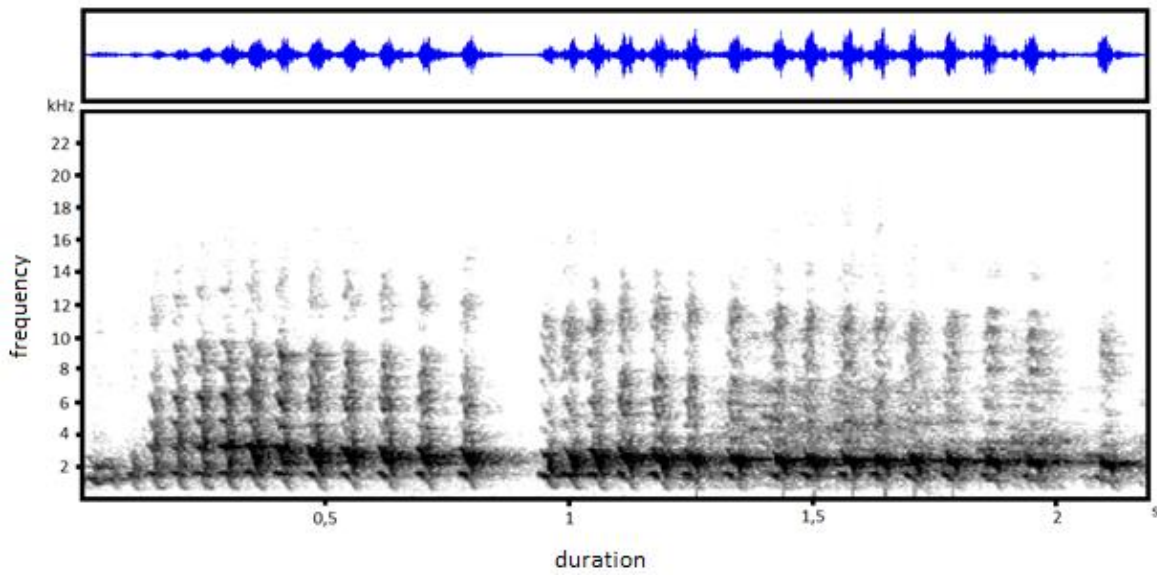


Fig. 10. Spectrogram and waveform of contact song - female.

Tab. 18. Description of measured acoustic parameters of contact song used for the statistical analysis.

<b>Contact song parameters</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Std.Dev.</b>
Duration	3,059	0,410	10,130	2,163
M elements	4,333	0,000	22,000	5,677
F trill cadency	3,127	0,000	11,000	3,554
Q1 Freq (Hz)	1746,281	1359,400	2671,900	289,815
Q1 Time (s)	34,825	3,000	82,200	19,472
Q3 Freq (Hz)	2293,159	1500,000	4500,000	563,400
Q3 Time (s)	35,914	3,800	83,500	19,551
Agg Entropy (u)	4,570	3,035	6,529	0,783
BW 90% (Hz)	1426,340	328,100	5015,600	990,573
Dur 90% (s)	2,384	0,300	7,800	1,595
Freq 5% (Hz)	1467,263	1125,000	1968,800	210,247
Freq 95% (Hz)	2893,602	1781,200	6421,900	947,997
IQR BW (Hz)	546,878	93,800	2531,200	485,246
IQR Dur (s)	1,078	0,100	4,200	0,806
Time 5% (s)	34,114	2,300	81,300	19,519
Time 95% (s)	36,490	4,300	84,400	19,612
Max Time (s)	35,352	3,298	83,328	19,553
Max Time %	56,661	3,456	95,944	24,603
Min Time (s)	35,402	3,298	82,406	19,531
Min Time %	57,672	3,425	95,944	24,699
Peak Time (s)	35,401	3,298	82,406	19,566
RMS Amp (u)	1382,208	156,100	5204,400	1160,382

Tab. 19. Correlations of measured parameters of contact song, red marked values are the most correlated ( $r \geq 0,9$ ) parameters.

	<b>Duration</b>	1,00	-0,29	-0,13	-0,32	-0,10	-0,10	-0,28	-0,22	0,65	-0,14	-0,09	-0,11	0,18	-0,11	0,13	-0,11	0,22
	<b>F trill cadency alone</b>	-0,29	1,00	0,49	-0,07	0,29	-0,05	0,00	0,11	-0,09	-0,06	-0,07	-0,08	-0,44	-0,08	-0,51	-0,08	0,03
	<b>Q1 Freq (Hz)</b>	-0,26	0,49	1,00	0,15	0,51	0,14	0,34	-0,01	-0,12	0,15	0,14	0,15	-0,01	0,14	-0,07	0,14	0,08
	<b>Q1 Time (s)</b>	-0,13	-0,07	0,15	1,00	0,22	1,00	0,18	0,17	0,08	1,00	1,00	1,00	0,03	1,00	-0,05	1,00	-0,09
	<b>Q3 Freq (Hz)</b>	-0,32	0,29	0,51	0,22	1,00	0,21	0,85	0,86	-0,25	0,22	0,20	0,21	-0,17	0,21	-0,19	0,21	-0,24
	<b>Q3 Time (s)</b>	-0,10	-0,07	0,14	1,00	0,21	1,00	0,16	0,16	0,12	1,00	1,00	1,00	0,02	1,00	-0,05	1,00	-0,07
	<b>Agg Entropy (u)</b>	-0,10	-0,05	0,15	0,26	0,76	0,25	0,86	0,80	1,00	0,82	0,26	0,25	-0,06	0,26	-0,06	0,26	-0,43
	<b>BW 90% (Hz)</b>	-0,25	0,00	0,18	0,17	0,81	0,15	0,83	0,83	0,86	-0,32	0,17	0,14	-0,02	0,16	0,01	0,16	-0,44
	<b>Dur 90% (s)</b>	0,67	-0,10	-0,12	0,04	-0,27	0,08	-0,32	-0,24	1,00	0,06	0,02	0,10	0,05	-0,12	0,06	-0,09	0,46
	<b>Freq 5% (Hz)</b>	-0,10	0,49	0,71	0,01	0,04	0,01	1,00	-0,10	-0,38	0,06	0,01	0,02	-0,11	0,01	-0,15	0,00	0,42
	<b>Freq 95% (Hz)</b>	-0,28	0,11	0,34	0,18	0,85	0,16	0,82	0,79	-0,30	-0,32	0,18	0,15	0,17	-0,05	0,17	-0,02	-0,37
	<b>IQR BW (Hz)</b>	-0,22	0,05	-0,01	0,17	0,86	0,16	0,83	0,79	1,00	-0,24	0,17	0,15	0,16	-0,19	0,16	-0,19	-0,33
	<b>IQR Dur (s)</b>	0,65	-0,09	-0,12	0,08	-0,25	0,12	-0,14	-0,30	-0,23	1,00	0,06	0,14	0,09	-0,19	0,10	-0,15	0,51
	<b>Time 5% (s)</b>	-0,14	-0,06	0,15	1,00	0,22	1,00	0,26	0,17	0,06	1,00	1,00	1,00	0,02	1,00	-0,05	1,00	-0,10
	<b>Time 95% (s)</b>	-0,09	-0,07	0,14	1,00	0,20	1,00	0,25	0,15	0,14	1,00	1,00	1,00	0,01	1,00	-0,06	1,00	-0,06
	<b>Max Time (s)</b>	-0,11	-0,08	0,15	1,00	0,21	1,00	0,26	0,16	0,09	1,00	1,00	1,00	0,05	1,00	-0,03	1,00	-0,09
	<b>Max Time %</b>	0,18	-0,44	-0,01	0,03	-0,17	0,02	-0,06	-0,19	-0,19	0,02	0,01	0,05	1,00	0,04	0,89	0,04	-0,15
	<b>Min Time (s)</b>	-0,11	-0,08	0,14	1,00	0,21	1,00	0,26	0,16	0,10	1,00	1,00	1,00	0,04	1,00	-0,02	1,00	-0,08
	<b>Min Time %</b>	0,13	-0,51	-0,07	-0,05	-0,19	-0,05	-0,02	-0,19	-0,15	-0,05	-0,06	-0,03	0,89	-0,02	1,00	-0,02	-0,01
	<b>Peak Time (s)</b>	-0,11	-0,08	0,14	1,00	0,21	1,00	0,26	0,16	0,10	1,00	1,00	1,00	0,04	1,00	-0,02	1,00	-0,08
	<b>RMS Amp (u)</b>	0,22	0,03	0,08	-0,09	-0,24	-0,07	-0,43	-0,37	-0,33	0,51	-0,10	-0,06	-0,09	-0,15	-0,08	-0,01	-0,08

## Duet

The duet was the vocalization, which were expressed the most frequently during the whole recording. It occurred only in pairs and in adult individuals of the opposite sex who were in the vicinity (in different enclosures) and later of which new pair were formed according to singing the duet. The individuals expressed this vocalization mainly in response to some distraction as approach of the keeper and me or presence of the cat and they also responded to duet vocalization of other individuals. It pointed to greater disruption of individuals at any given moment of that vocalization. The following table (Tab.20) describes the representation of the duet according to sex and context. Duration of duet reached 2,3 – 17,12 s ( $5,52 \pm 2,74$ ) (see Tab.21). For the spectrogram of duet see Fig.11. The description of all measured acoustic parameters of trill used in the statistic is in Tab.22 and the correlation of those parameters is represented in Tab.23.

Tab. 20. The description of the duet: representation of sex and context.

DUET	Sex		Context					
	Male	Female	Answer	Approach	Cat	Standard	Bonding	Observing
<b>Number of Vocalization</b>	242	242	38	34	10	400	2	0
<b>Percentage</b>	50	50	7,9	7	2,1	82,6	0,4	0

Tab. 21. Descriptive statistic of the duet: representation of the duration range (mean, maximum, minimum and standard deviation) among all recorded individuals.

Variable	Mean	Minimum	Maximum	Std.Dev.
<b>Duration</b>	5,52	2,3	17,12	2,74

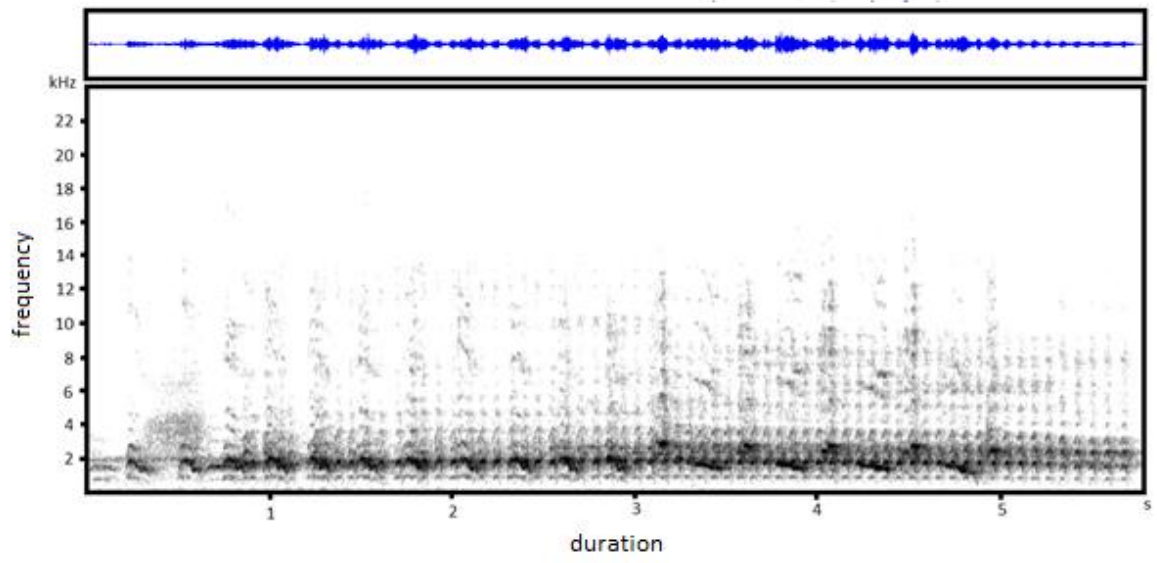


Fig. 11. Spectrogram and waveform of duet.



Tab. 22. Description of measured acoustic parameters of duet used for the statistical analysis.

<b>Duet parameters</b>	<b>Mean</b>	<b>Minimum</b>	<b>Maximum</b>	<b>Std.Dev.</b>
duet duration	5,534	2,300	17,12	2,748
Male%	78,654	34,000	100,00	13,422
Duration Female	5,156	0,630	16,88	2,802
Duration M1	3,886	0,330	17,12	2,293
Duration M2	0,411	0,000	6,91	1,131
Start time male	0,056	-4,330	2,26	0,808
Duration of twitter part M	1,777	0,000	6,08	1,327
interval M1 - M2	0,164	0,000	3,92	0,544
M elements	11,110	2,000	38,00	6,201
F trill cadency	6,714	5,000	8,00	0,733
High Freq	9508,912	3157,000	14330,00	2476,389
Low Freq	674,846	501,000	902,00	103,369
Freq Range	8833,956	2305,000	13829,00	2525,514
Q1 Freq (Hz)	1755,492	1406,200	2296,90	151,754
Q1 Time (s)	34,565	1,500	92,30	21,481
Q3 Freq (Hz)	2215,487	1875,000	2906,20	187,088
Q3 Time (s)	36,445	3,400	93,60	21,599
Agg Entropy (u)	4,853	3,971	5,87	0,392
BW 90% (Hz)	1382,557	562,500	3703,10	577,714
Dur 90% (s)	3,793	1,500	15,10	2,100
Freq 5% (Hz)	1478,368	1218,800	1828,10	123,934
Freq 95% (Hz)	2860,916	2250,000	5062,50	566,814
IQR BW (Hz)	459,987	187,500	1078,10	202,320
IQR Dur (s)	1,870	0,400	9,70	1,355
Time 5% (s)	33,634	0,600	91,70	21,405
Time 95% (s)	37,419	3,800	95,90	21,622
Max Time (s)	35,542	1,324	92,28	21,252
Max Time %	54,186	13,998	93,57	19,640
Min Time (s)	35,475	1,390	92,28	21,401
Min Time %	53,472	7,110	93,60	19,336
Peak Time (s)	35,643	1,324	92,28	21,378
RMS Amp (u)	2068,807	522,500	4774,20	1006,654



## 5.2 Social type and context

At first I tested whether the social type and context had any significant influence on expressed calls. In the calls where was not possible to test both of these categories (twitter, duet), because some types of the category was under-represented, I tested just one category. For each call type was significant effect of social type and context in at least one of the call parameters.

### Twitter

Because of the variability of representatives in context was low, I tested only the effect of social type on twitter. From 20 measured parameters, in two was confirmed the significant influence after Bonferroni correction ( $p \leq 0,001$ ): Frequency 95% and Aggregate Entropy. I found that the twitter parameter of Frequency 95% reached the highest values when pair expressed twitter: One-way ANOVA:  $F(3, 113) = 6.2370$ ,  $p < 0,001$ . Post-hoc test showed that the most significant differences are between pair and two males ( $p < 0,05$ ) and between pair and female in separate enclosure ( $p < 0,01$ ) (Fig.12).

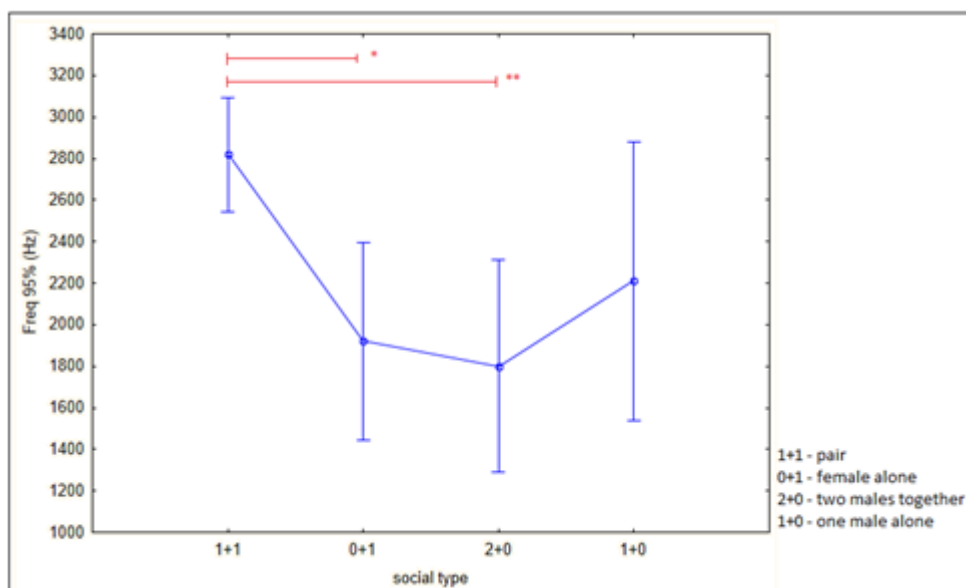


Fig. 12. Influence of social type on twitter in the parameter of Frequency 95% (Hz) (the frequency that divides the selection into two frequency intervals containing 95% and 5% of the energy in the selection, the summed energy has to exceed 95% of the total energy). The significant differences were between pair and two males, and between pair and female in separate enclosure (\*  $p < 0,05$ , \*\*  $p < 0,01$ ).

## Trill

I tested whether the social type and context affected the expression of trill. Of all measured parameters only RMS Amplitude had significant influence (context:  $p < 0,001$ , social type:  $p < 0,001$  and the interaction:  $p < 0,001$ ).

In social type I included two representatives into other categories, because of its low representation for the analysis (2+0 into 1+0, 1+1+ into 1+1). I found a significant influence of social type, when trill was the loudest (RMS Amplitude) in situation when females were housed alone: Factorial ANOVA:  $F(2, 123) = 43.199$ ,  $p < 0,001$ . The most significant difference was between female (in separate enclosure) and pair according to post-hoc test (see Fig.13).

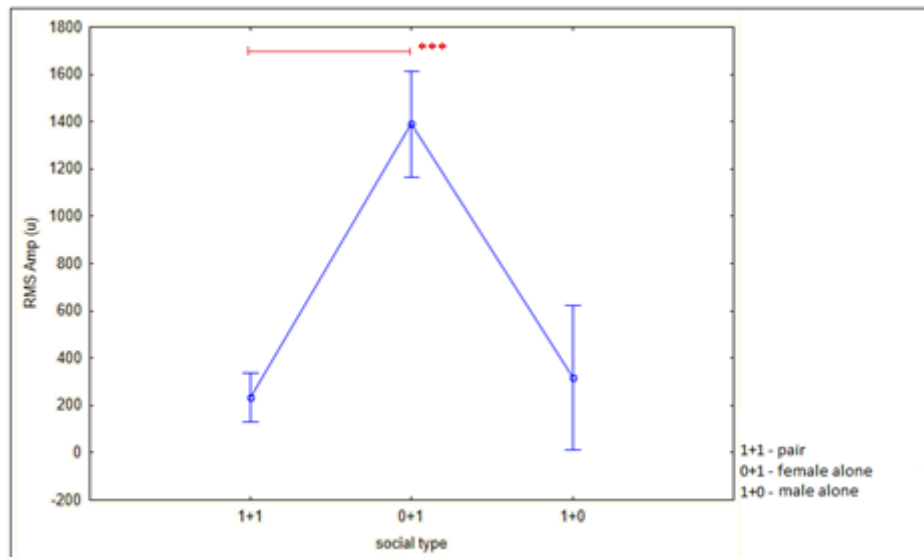


Fig. 13. Influence of social type on trill in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal). The most significant difference was between pair and female (\*\*\*)  $p < 0,001$ ).

For testing of context two situations were excluded, because of its small sample size (answer:  $n = 1$ , cat:  $n = 1$ ). RMS Amplitude of trill was higher during the approach of the keeper or me: Factorial ANOVA:  $F(1, 123) = 18,132$ ,  $p < 0,001$  (see Fig.14).

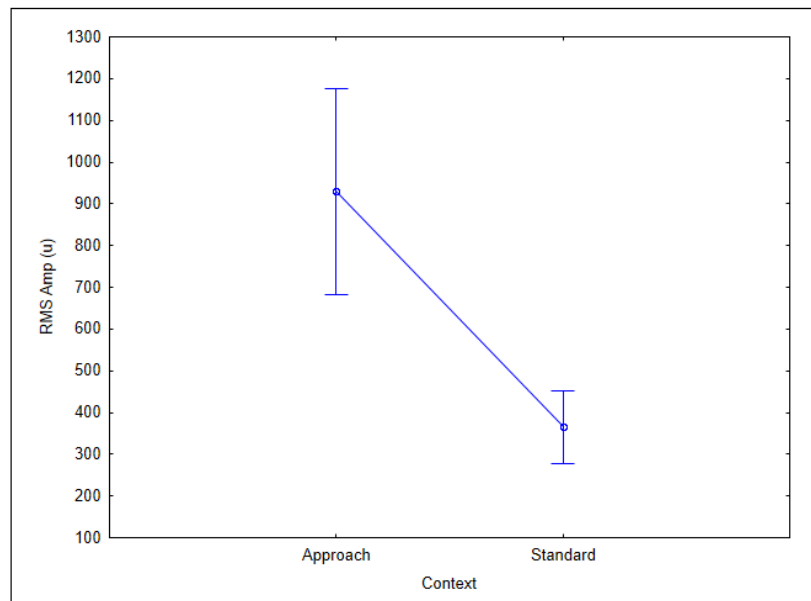


Fig. 14. Influence of context on trill in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal).

### Contact song

I examined the influence of social type and context on contact song. For analysing of social type I included two smaller categories (2+0, 1+1+:  $n = 3$ ) into those which had more representatives (2+0 into 1+0, 1+1+ into 1+1). For testing of context I put three situations (approach:  $n = 2$ , bonding:  $n = 4$ , answer:  $n=7$ ) together into one category *other*, because its separate sample size was too small for the analysis.

From 22 parameters six were significant ( $p < 0,05$ ) for one of the categories or its interaction: Duration, F trill cadency, 1st Quartile Frequency, Aggregate Entropy, Frequency 5% and RMS Amplitude. The social type had significant influence after the Bonferroni correction in three parameters of contact song: F trill cadency, Frequency 5% and RMS Amplitude. The influence of context was significant ( $p = 0,02$ ) only in one parameter (RMS Amplitude).

I discovered a significant influence, when the contact song was the loudest (RMS Amplitude) when some distraction from keeper or me appeared or when the individuals reacted to each other and it was under the conditions when the females were housed alone in the enclosure: Factorial ANOVA:  $F(2, 57) = 3.4057$ ,  $p = 0,04$ . The post-hoc test showed that the most significant differences were between pair and female in separate enclosure and between male and female (both in separate enclosures) (Fig.15).

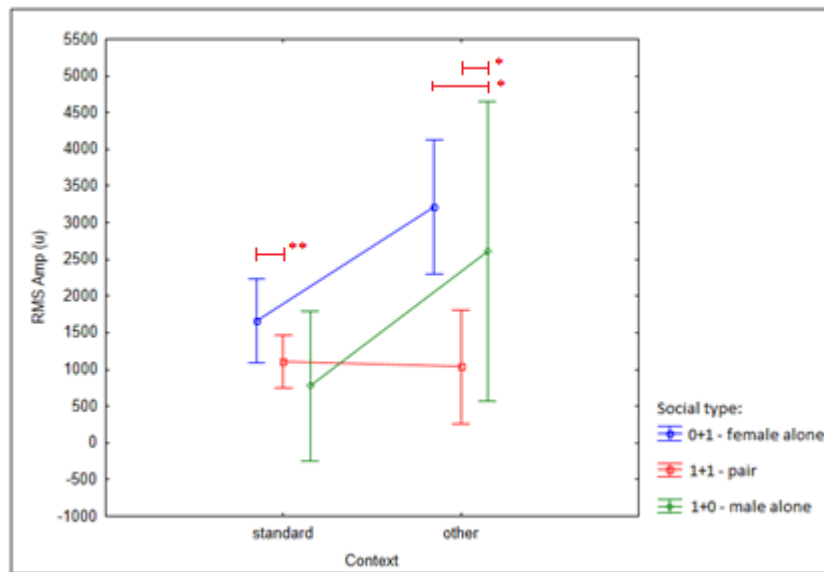


Fig. 15. Influence of social type and context on contact song in the parameter of RMS Amplitude (the root-mean-square amplitude of the selected part of the signal). The most significant differences were between pair and female housed alone in standard situation (\*\* $p < 0,01$ ) and in other situation, when the keeper approached to the enclosures or when the individuals responded to each other (\* $p < 0,05$ ). The significant difference were also between female and male (both in separate enclosures) in standard situation (\* $p < 0,05$ ).

## Duet

In 32 parameters I tested whether the social type and context affected the expression of duet. The social type had Bonferroni corrected significant influence ( $p \leq 0,001$ ) in one parameter - Frequency Range. The context significantly ( $p \leq 0,001$ ) affected the duet in five parameters: Duet Duration, Duration Female, Interval M1 – M2, Duration 90% and IQR Duration.

I found a significant influence of social type, when duet had the lowest frequency range in the situation when the pair was housed in the enclosure with hatched chicks (One-way ANOVA:  $F(3, 178) = 9.8101, p < 0,001$ ). Post-hoc test showed that the most significant difference was between pair and pair with hatched chicks ( $p < 0,001$ ), and between pair with hatched chicks and female and male (in separate enclosures) ( $p < 0,05$ ) (see Fig. 16).

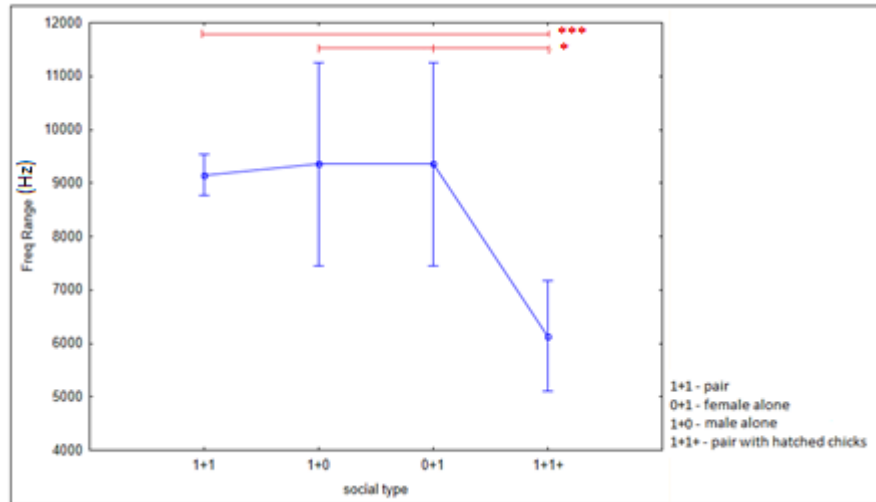


Fig. 16. Influence of social type on frequency range (Hz) (the difference between the highest and lowest frequency) in duet. The significant influence was between pair and pair with hatched chicks and between female and male in separate enclosures in comparison with pair with hatched chicks (\*  $p < 0,05$ , \*\*\*  $p < 0,001$ ).

I discovered that the context significantly affected the duet (especially the duration parameters) when the keeper or me approached the enclosures or were nearby. In this situation the duration of the duet was longer (One-way ANOVA:  $F(2, 171) = 8.0808$ ,  $p < 0,001$ ). The most significant differences were according to post-hoc test between the contexts approach and standard, and approach and answer (see Fig. 17).

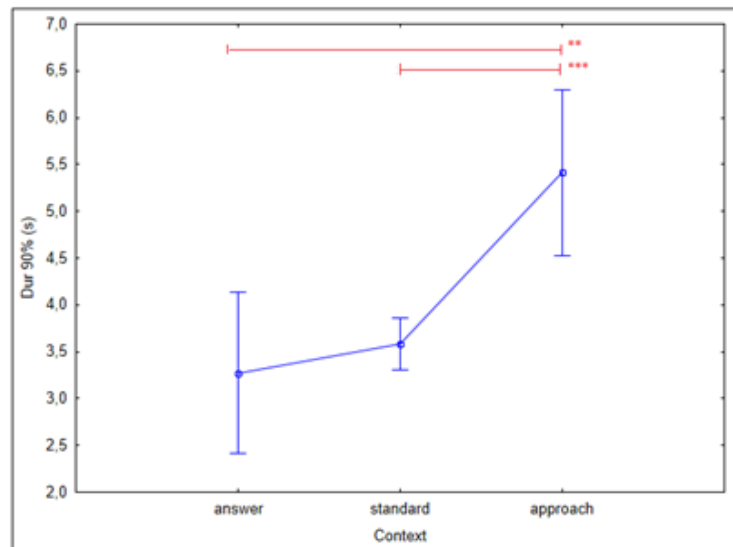


Fig. 17. Influence of context on duet in the parameter Dur 90% (s) (the difference between the 5% and 95% times). The most significant differences were between approach and answer and between approach and standard (\*\*  $p < 0,01$ , \*\*\*  $p < 0,001$ ).

### 5.3 Sex and other categories

Furthermore I tested whether the sex and its interaction with social type and context had any effect on the manifested calls. The impact of sex was found in two call types: contact song and duet.

#### Contact song

I tested whether the sex had an impact on contact song within 22 measured parameters. In three parameters was after Bonferroni correction confirmed significant difference ( $p \leq 0,002$ ) between the sex: 1st Quartile Frequency, Frequency 5% and Max Time %.

I found that females reached higher frequencies in the 1st Quartile Frequency of the amplitude: Mann-Whitney U Test:  $p < 0,002$  (Fig. 18).



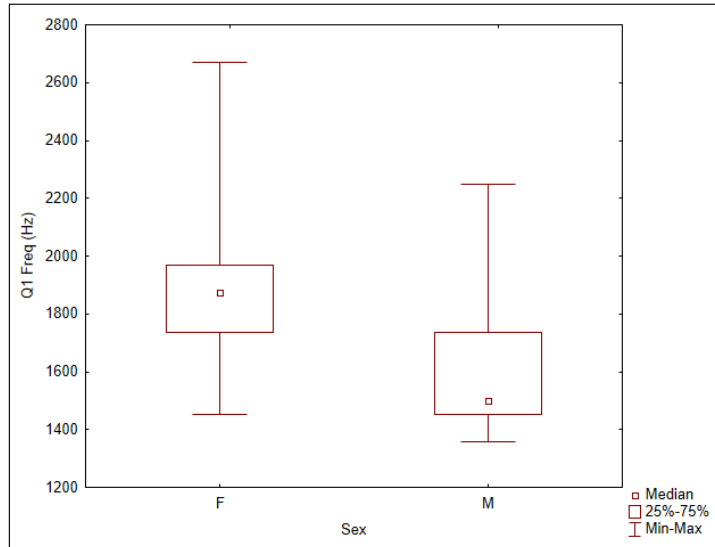


Fig. 18. The difference between female (F) and male (M) in the parameter 1st Quartile Frequency (Hz) (the frequency that divides the selection into two frequency intervals containing 25% and 75% of the energy in the selection, the summed energy has to exceed 25% of the total energy) of contact song.

## Duet

Within 32 measured parameters, I examined if the sex of the individual who initiated the duet and its interaction with social type and context had effect on duet expression. The Bonferroni corrected significant influence ( $p \leq 0,001$ ) of the sex of the individual who initiated the duet with the interaction of social type was shown in two parameters: Aggregate Entropy and Bandwidth 90%.

I found out that the disorder of the sound (Aggregate Entropy) was highest in the situation when the male started to express the duet: Factorial ANOVA:  $F(2, 170) = 9.6956$ ,  $p < 0,001$ . Post-hoc test showed that the most significant differences were between situations when both sex started to sing at the same time and when female started to sing duet, and between both sex and male who initiated duet (Fig. 19).

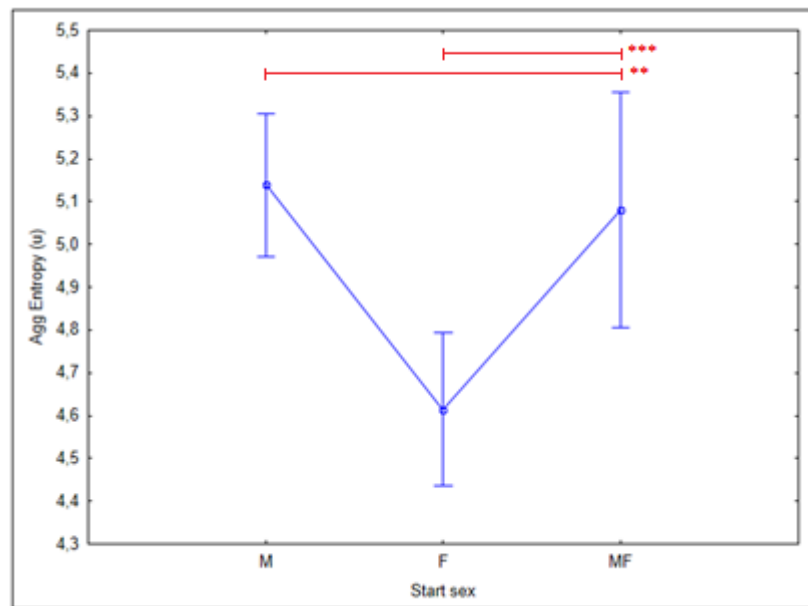


Fig. 19. Influence of the sex of individual who initiated duet on the Aggregate Entropy (the aggregate entropy measures the disorder in a sound by analysing the energy distribution within a selection, higher entropy values correspond to greater disorder in the sound whereas a pure tone would have zero entropy) of duet. The most significant difference was between MF (when the individuals started the duet together) and F (when the female started to sing duet) (\*\* $p < 0,001$ ) and between MF and M (when the male started to sing duet) (\*\* $p < 0,01$ ).

For the interaction with context I put the two situations answer and approach into one category *other* to have bigger sample for comparison to standard situation for the analysis. However, the influence of context and its interaction with start sex have not shown any significant value. The influence of sex of the individual who initiated duet was significant after Bonferroni correction ( $p \leq 0,001$ ) in three parameters: Duration M1, Frequency 95% and Bandwidth 90%.

I discovered that the duration of the 1st male part of the duet was significantly longer when males started to express duet: Factorial ANOVA:  $F(2, 176) = 8.4883$ ,  $p < 0,001$ ). According to post-hoc test the main differences of sex starting the duet were between female and male in both context situations, and between male and both sex in standard situation (Fig. 20).

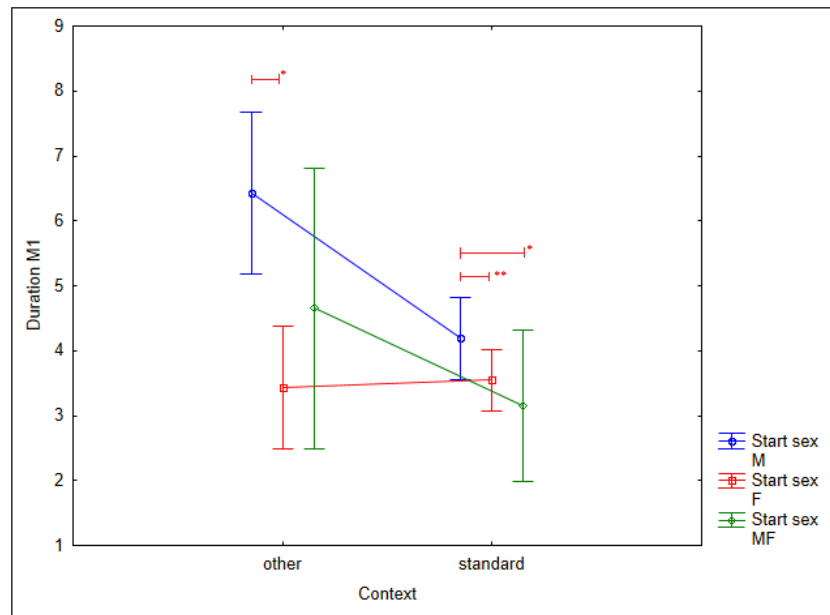


Fig. 20. Influence of the sex of individual who initiated duet on the duration of the 1st male part (s) of duet in the standard context situation and when the individuals were affected by approach of keeper or when they reacted to each other. Differences of sex of the individual who started to sing duet were the most significant between female and male in standard situation (\*\* $p < 0,01$ ) and in the other situations (approach, answer) (\* $p < 0,05$ ), and between male and both sex in standard situation (\* $p < 0,05$ ).

## 5.4 Individual variability

### 5.4.1 Univariate statistics and Potential of Individuality Coding (PIC)

Further I tested whether there were disparities between the individuals in different types of calls within the measured parameters. At first I used simple univariate statistics (one-way ANOVA) for testing the variability between individuals. Second I calculated the PIC value for each parameter of expressed calls using the coefficients of variance (CV) of variability within and among individuals ( $PIC = CV_A / \text{mean } CV_W$ ).

### Twitter

With a one-way ANOVA I examined the influence of each individual on the values of the variables of twitter. After the Bonferroni correction seven parameters showed significant differences ( $p \leq 0,002$ ) between the individuals among the 20 measured twitter parameters: 1st Quartile Frequency, 3rd Quartile Frequency, Aggregate Entropy, Bandwidth 90%, Frequency 95%, IQR Bandwidth and RMS Amplitude.

The following table (Tab. 24) describes the results of one-way ANOVA for twitter and its PIC in the measured parameters.

Tab. 24. Descriptive statistics (mean  $\pm$  SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of twitter and the results of the calculation of their PIC.

Twitter parameters	Mean $\pm$ SD (N = 117)	p-value*	CV <sub>A</sub>	Mean CV <sub>W</sub>	PIC
Duration	0,14 $\pm$ 0,11	-	79,1	36,6	2,2
Q1 Freq (Hz)	1436,70 $\pm$ 315,61	0,0017*	22,0	17,4	1,3
Q1 Time (s)	33,24 $\pm$ 19,63	-	59,0	49,0	1,2
Q3 Freq (Hz)	1949,52 $\pm$ 834,57	<0,001*	42,8	24,0	1,8
Q3 Time (s)	33,27 $\pm$ 19,63	-	59,0	48,9	1,2
Agg Entropy (u)	4,10 $\pm$ 0,94	<0,001*	23,0	14,9	1,5
BW 90% (Hz)	1365,38 $\pm$ 1154,78	<0,001*	84,6	48,5	1,7
Dur 90% (s)	0,09 $\pm$ 0,10	-	110,8	57,0	1,9
Freq 5% (Hz)	1062,10 $\pm$ 329,47	-	31,0	22,4	1,4
Freq 95% (Hz)	2427,49 $\pm$ 1196,82	<0,001*	49,3	26,1	1,9
IQR BW (Hz)	512,82 $\pm$ 699,16	<0,001*	136,3	63,4	2,1
IQR Dur (s)	0,01 $\pm$ 0,05	-	394,8	102,7	3,8
Time 5% (s)	33,22 $\pm$ 19,62	-	59,1	49,0	1,2
Time 95% (s)	33,31 $\pm$ 19,63	-	58,9	48,9	1,2
Max Time (s)	33,25 $\pm$ 19,63	-	59,0	48,9	1,2
Max Time %	36,92 $\pm$ 14,66	-	39,7	29,0	1,4
Min Time (s)	33,25 $\pm$ 19,62	-	59,0	48,9	1,2
Min Time %	36,75 $\pm$ 14,15	-	38,5	27,8	1,4
Peak Time (s)	33,25 $\pm$ 19,62	-	59,0	48,9	1,2
RMS Amp (u)	316,04 $\pm$ 437,84	-	138,5	48,6	2,8

\* significant p-values (Bonferroni corrected)

CV<sub>A</sub> - coefficient of variation among individuals

mean CV<sub>W</sub> - mean of the coefficients of variation within individuals

PIC - Potential of Individuality Coding

## Trill

I tested whether the individuals affected the expression of trill. In nine parameters (from 21 measured) was confirmed the Bonferroni corrected significant influence ( $p \leq 0,002$ ) of each individual on trill: 3rd Quartile Frequency, Aggregate Entropy, Bandwidth 90%, Frequency 5%, Frequency 95%, IQR Bandwidth, Max Time %, Min Time % and RMS Amplitude. For the results of ANOVA and PIC for trill parameters see Tab. 25.

Tab. 25. Descriptive statistics (mean  $\pm$  SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of trill and the results of the calculation of their PIC.

Trill parameters	Mean $\pm$ SD (120)	p-value*	CV <sub>A</sub>	Mean CV <sub>w</sub>	PIC
Duration	0,31 $\pm$ 0,23	-	74,0	43,9	1,7
Trill cadency	5,92 $\pm$ 3,69	-	62,3	46,4	1,3
Q1 Freq (Hz)	1876,46 $\pm$ 474,39	-	25,3	18,5	1,4
Q1 Time (s)	32,56 $\pm$ 20,31	-	62,4	53,2	1,2
Q3 Freq (Hz)	3302,33 $\pm$ 1096,67	<0,001*	33,2	18,7	1,8
Q3 Time (s)	32,66 $\pm$ 20,32	-	62,2	53,0	1,2
Agg Entropy (u)	5,83 $\pm$ 0,72	<0,001*	12,3	7,8	1,6
BW 90% (Hz)	3521,44 $\pm$ 1299,83	<0,001*	36,9	26,4	1,4
Dur 90% (s)	0,24 $\pm$ 0,19	-	77,7	52,9	1,5
Freq 5% (Hz)	1284,15 $\pm$ 241,90	0,0019*	18,8	14,5	1,3
Freq 95% (Hz)	4805,60 $\pm$ 1390,22	<0,001*	28,9	19,3	1,5
IQR BW (Hz)	1425,87 $\pm$ 818,75	<0,001*	57,4	36,8	1,6
IQR Dur (s)	0,10 $\pm$ 0,14	-	134,6	110,8	1,2
Time 5% (s)	32,53 $\pm$ 20,31	-	62,4	53,2	1,2
Time 95% (s)	32,76 $\pm$ 20,31	-	62,0	52,9	1,2
Max Time (s)	32,58 $\pm$ 20,31	-	62,3	53,2	1,2
Max Time %	25,05 $\pm$ 19,33	<0,001*	77,2	56,6	1,4
Min Time (s)	32,58 $\pm$ 20,30	-	62,3	53,2	1,2
Min Time %	24,99 $\pm$ 20,20	<0,001*	80,8	59,4	1,4
Peak Time (s)	32,58 $\pm$ 20,30	-	62,3	53,1	1,2
RMS Amp (u)	290,18 $\pm$ 401,32	<0,001*	138,3	39,2	3,5

\* significant p-values (Bonferroni corrected)

CV<sub>A</sub> - coefficient of variation among individuals

mean CV<sub>w</sub> - mean of the coefficients of variation within individuals

PIC - Potential of Individuality Coding

### Contact song

I also tested the influence of each individual on the measured parameters of contact song. The effect of the individuality was significant after Bonferroni correction ( $p \leq 0,002$ ) in two from 20 parameters: Frequency 5% and Min Time %. The result of ANOVA and the PIC of contact song parameters is presented in Tab. 26.

Tab. 26. Descriptive statistics (mean  $\pm$  SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of contact song and the results of the calculation of their PIC.

Contact song parameters	Mean $\pm$ SD (N = 63)	p-value*	CV <sub>A</sub>	mean CV <sub>W</sub>	PIC
Duration	3,16 $\pm$ 2,11	-	66,7	45,1	1,5
M elements	4,33 $\pm$ 5,68	-	131,0	24,0	5,5
F trill cadency alone	3,13 $\pm$ 3,55	-	113,6	8,0	14,1
Q1 Freq (Hz)	1746,28 $\pm$ 289,81	-	16,6	11,1	1,5
Q1 Time (s)	34,83 $\pm$ 19,47	-	55,9	40,0	1,4
Q3 Freq (Hz)	2293,16 $\pm$ 563,40	-	24,6	16,1	1,5
Q3 Time (s)	35,91 $\pm$ 19,55	-	54,4	39,3	1,4
Agg Entropy (u)	4,57 $\pm$ 0,78	-	17,1	11,3	1,5
BW 90% (Hz)	1426,34 $\pm$ 990,57	-	69,4	35,9	1,9
Dur 90% (s)	2,38 $\pm$ 1,60	-	66,9	50,6	1,3
Freq 5% (Hz)	1467,26 $\pm$ 210,25	<0,001*	14,3	7,2	2,0
Freq 95% (Hz)	2893,60 $\pm$ 948,00	-	32,8	18,6	1,8
IQR BW (Hz)	546,88 $\pm$ 485,25	-	88,7	51,8	1,7
IQR Dur (s)	1,08 $\pm$ 0,81	-	74,8	56,2	1,3
Time 5% (s)	34,11 $\pm$ 19,52	-	57,2	40,8	1,4
Time 95% (s)	36,49 $\pm$ 19,61	-	53,7	38,8	1,4
Max Time (s)	35,35 $\pm$ 19,55	-	55,3	39,8	1,4
Max Time %	56,66 $\pm$ 24,60	-	43,4	41,7	1,0
Min Time (s)	35,40 $\pm$ 19,53	-	55,2	39,8	1,4
Min Time %	57,67 $\pm$ 24,70	<0,001*	42,8	38,2	1,1
Peak Time (s)	35,40 $\pm$ 19,57	-	55,3	39,8	1,4
RMS Amp (u)	1382,21 $\pm$ 1160,38	-	84,0	59,7	1,4

\* significant p-values (Bonferroni corrected)

CV<sub>A</sub> - coefficient of variation among individuals

mean CV<sub>W</sub> - mean of the coefficients of variation within individuals

PIC - Potential of Individuality Coding

## Duet

I examined whether there was a difference between the pairs in the measured parameters of duet. From 32 variables in 11 were Bonferroni corrected significant variance ( $p \leq 0,001$ ) of pairs: Duration, Male %, Duration Female, M elements, F Trill Cadency, High Frequency, Frequency Range, 1st Quartile Frequency, Frequency 5%, IQR Bandwidth and RMS Amplitude. In Tab. 27 are presented the results of ANOVA and PIC of duet parameters.

I found that in duration of duet was significant difference between pairs: One-way ANOVA:  $F(9, 81) = 3.5581, p < 0,001$  (Fig. 21).

Tab. 27. Descriptive statistics (mean  $\pm$  SD) and univariate analysis of variance (one-way ANOVA) results showing the influence of individuals on the measured parameters of duet and the results of the calculation of their PIC.

Duet parameters	Mean $\pm$ SD (N = 92)	p-value*	CV <sub>A</sub>	mean CV <sub>W</sub>	PIC
duet duration	5,53 $\pm$ 2,75	<0,001*	49,7	34,5	1,4
Male%	78,65 $\pm$ 13,42	<0,001*	17,1	18,1	0,9
Duration Female	5,16 $\pm$ 2,80	<0,001*	54,4	36,6	1,5
Duration M1	3,89 $\pm$ 2,29	-	59,0	48,2	1,2
Duration M2	0,41 $\pm$ 1,13	-	275,3	125,6	2,2
Start time male	0,06 $\pm$ 0,81	-	1442,5	114,4	12,6
Duration of twitter part M	1,78 $\pm$ 1,33	-	74,7	75,8	1,0
interval M1 - M2	0,16 $\pm$ 0,54	-	331,6	121,0	2,7
M elements	11,11 $\pm$ 6,20	<0,001*	55,8	40,8	1,4
F trill cadency duet	6,71 $\pm$ 0,73	<0,001*	10,9	8,7	1,3
High Freq	9508 $\pm$ 2476,39	<0,001*	26,0	20,4	1,3
Low Freq	674,85 $\pm$ 103,37	-	15,3	13,8	1,1
Freq Range	8833,96 $\pm$ 2525,51	<0,001*	28,6	22,8	1,3
Q1 Freq (Hz)	1755,49 $\pm$ 151,75	<0,001*	8,6	5,9	1,5
Q1 Time (s)	34,56 $\pm$ 21,48	-	62,1	62,2	1,0
Q3 Freq (Hz)	2215,49 $\pm$ 187,09	-	8,4	7,3	1,2
Q3 Time (s)	36,45 $\pm$ 21,60	-	59,3	58,9	1,0
Agg Entropy (u)	4,85 $\pm$ 0,39	-	8,1	8,3	1,0
BW 90% (Hz)	1382,56 $\pm$ 577,71	-	41,8	38,1	1,1
Dur 90% (s)	3,79 $\pm$ 2,10	-	55,4	36,1	1,5
Freq 5% (Hz)	1478,37 $\pm$ 123,93	<0,001*	8,4	6,7	1,2
Freq 95% (Hz)	2860,92 $\pm$ 566,81	-	19,8	18,1	1,1
IQR BW (Hz)	459,99 $\pm$ 202,32	<0,001*	44,0	35,8	1,2
IQR Dur (s)	1,87 $\pm$ 1,36	-	72,5	44,2	1,6
Time 5% (s)	33,63 $\pm$ 21,40	-	63,6	64,2	1,0
Time 95% (s)	37,42 $\pm$ 21,62	-	57,8	57,2	1,0
Max Time (s)	35,54 $\pm$ 21,25	-	59,8	59,8	1,0
Max Time %	54,19 $\pm$ 19,64	-	36,2	31,4	1,2
Min Time (s)	35,48 $\pm$ 21,40	-	60,3	60,1	1,0
Min Time %	53,47 $\pm$ 19,34	-	36,2	32,9	1,1
Peak Time (s)	35,64 $\pm$ 21,38	-	60,0	59,9	1,0
RMS Amp (u)	2068,81 $\pm$ 1006,65	<0,001*	48,7	35,3	1,4

\* significant p-values (Bonferroni corrected)

CV<sub>A</sub> - coefficient of variation among individuals

mean CV<sub>W</sub> - mean of the coefficients of variation within individuals

PIC - Potential of Individuality Coding

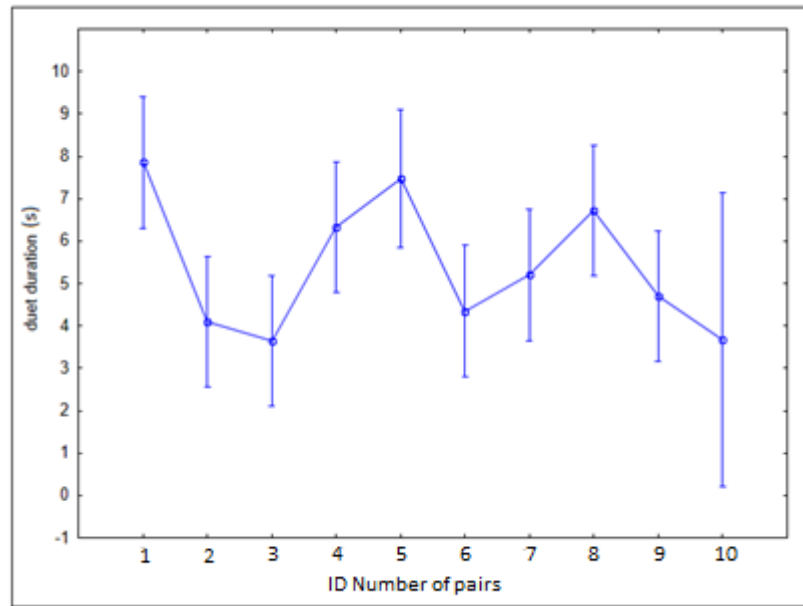


Fig. 21. Difference between pairs in the duration (s) of duet.

#### 5.4.2 Nonparametric test of variation coefficients

Using the Friedman test I examined whether the individuality within and among individuals differ over the call types (Fig.22). There was a statistically significant difference in inter-individuality among the expressed calls,  $\chi^2(3, 24) = 72.0, p < 0,001$ . Intra-individual variation did not differ among calls,  $\chi^2(3, 24) = 5.422, p = 0,143$ .

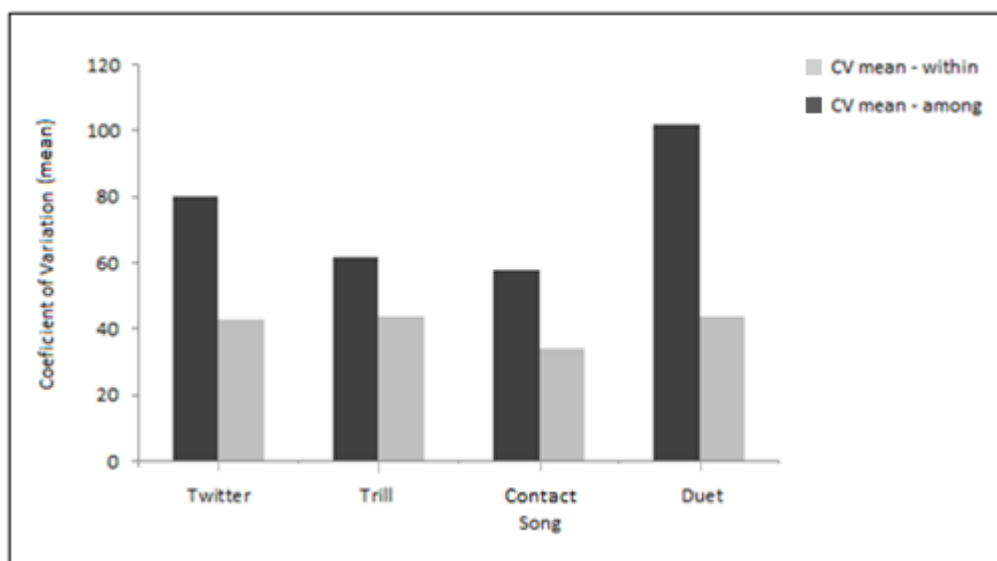


Fig. 22. Inter- and intra-individual mean coefficients of variation for each call type of the repertoire of Black-and-white Laughingthrush: Twitter, Trill, Song and Duet.



### 5.4.3 Discriminant Function Analysis (DFA)

The discriminant function analysis (DFA) was used to test whether individuals could be correctly classified by the call type parameters. The DFA was computed only for duets, because the sample size of other call types was smaller and the statistical software did not calculate them (Twitter:  $N = 117, 8,1 \pm 3,65$ ; Trill:  $N = 120, 8,53 \pm 1,9$ ; Contact song:  $N = 63, 6,87 \pm 3,52$ ).

Five pairs were included into analysis representing 95 duets. The discriminant function used seven from 32 duet parameters to correctly match pair's duet: Duration Female, Duration of Twitter part M, F Trill Cadency, 1st Quartile Frequency, Frequency 5%, Frequency 95% and RMS amplitude. DFA showed that 75,8% of duets were correctly classified to appropriate pair that expressed it and the validated value was 66,3% (Wilks' lambda = 0,146). In Tab. 28 are presented the values of prior probability of correct matching by chance in comparison with the correct matching after discriminant analysis. For the classification result of each pair see Tab. 29. The analysis generated four significant discriminant functions that included all seven parameters. The first function consisted of 44,1% of variation, the second function 21,4%, the third function 18,1% and the fourth function 16,4%. With the first discriminant function mostly correlated 1st Quartile Frequency ( $r = 0,564$ ) and Frequency 5% ( $r = 0,470$ ). With the second discriminant function mostly correlated Duration Female ( $r = 0,566$ ) and Duration of Twitter part M ( $r = 0,303$ ). I plotted the first two function scores (describing 66% of the variation) for duet with a centroid of all duet related to each pair with the spectrograms of each pair's duet in Fig. 23.

Tab. 28. Comparison of prior probability of correctly matched pairs (expressing duet) by chance with correctly classified pairs after conventional and validated discriminant analysis.

ID Number of pairs	Number of duets	Prior probability by chance (%)	Conventional DFA (%)	Validated DFA (%)
1	16	16,8	75	56,3
2	19	20	73,7	52,6
4	20	21,1	85	80
5	20	21,1	65	65
6	20	21,1	80	75
		$\phi$ 20,02	$\phi$ 75,8	$\phi$ 66,3

Tab. 29. Classification results of discriminant function analysis. The bold values show the correctly classified pairs expressing duet by original discriminant function and after cross-validation.

	ID Number of pair	1	2	4	5	6	
<b>Original</b>	1	<b>75</b>	0	18,8	0	6,3	
	2	0	<b>73,7</b>	10,5	0	15,8	
	4	10	0	<b>85</b>	0	5	
	5	5	5	15	<b>65</b>	10	
	6	0	0	10	10	<b>80</b>	
		1	<b>56,3</b>	0	25	0	18,8
<b>Cross-validated</b>	2	5,3	<b>52,6</b>	15,8	5,3	21,1	
	4	10	0	<b>80</b>	5	5	
	5	5	5	15	<b>65</b>	10	
	6	0	5	10	10	<b>75</b>	
		1	<b>56,3</b>	0	25	0	18,8
		2	5,3	<b>52,6</b>	15,8	5,3	21,1

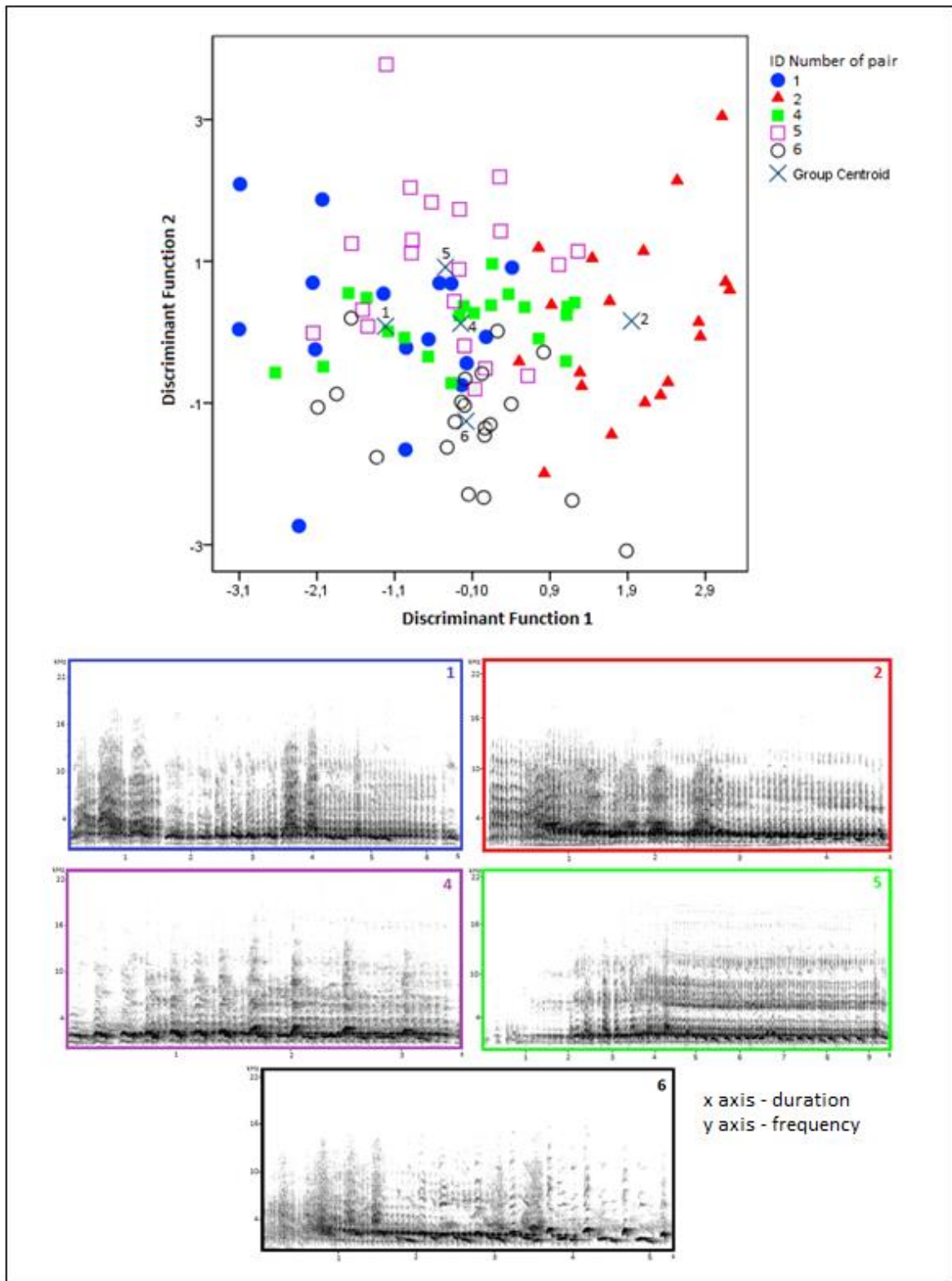


Fig. 23. Plot of five pair's duets of Black-and-white Laughingthrush on the space of the first two discriminant functions and the spectrogram of each pair's duet.

## 6 Discussion

The present research is the first detailed bioacoustic study on the individual identity of Black-and-white Laughingthrush. Similar studies on individual identity were also performed on other oscine species of South-East Asia as Brownish-flanked Warbler (*Horornis fortipes*) (Xia et al., 2010 and 2012), several studies on Zebra Finch (*Taeniopygia guttata*) (Vignal et al., 2008; Levréro et al., 2009; Reers et al., 2011) and Jungle Crow (*Corvus macrorhynchos*) (Kondo et al., 2010). It has been observed also in many other bird species that the vocalization is the most important clue to determine individual identity (Xia et al., 2012). If the vocal repertoire can be divided into different song types and elements and these are found in most individuals, the individual identity of the species may be successful (Xia et al., 2010).

### Repertoire

The results of this research showed that the repertoire includes four types of vocalization in both sex during nesting season: twitter, trill, contact song and duet. As I could observe from the behaviour of captive individuals, the vocalization was used especially for communication between some individuals and for territorial defense. To compare the vocal repertoire with other species, the best will be White-crested Laughingthrush (*Garrulax leucolophus*) because of the taxonomic history of these two species and similar social composition (formation of flock). Voice of White-crested Laughingthrush is described as a noisy and melodious laughing call (Collar and Robson, 2007). According to Chinkangsadarn (2012) *leucolophus* produces six vocal types: alert call, excitement call, invitation call, alarm call, and contact and mobbing subsongs. I compared the outcomes of his research with my results according to spectrograms or context of the vocalization types. Similar seems to be flying alert call of *leucolophus* (found when birds are moving to change their position over a short distance for example between branches) with trill, invitation call (*leucolophus*) with twitter, and contact subsong of *leucolophus* with contact song. Chinkangsadarn describes that this vocal repertoire is related to the social structure of the species, because they live in a flock and vocalization is used to social bonding and to protection of the group. I assume that the similar social behaviour and vocalization use will be presented in the wild population of Black-and-white Laughingthrush. The repertoire structure obtained from present research may not be

complete, because I recorded the acoustic vocalisation from the captive population and in a time period of one month. For more information about the singing behaviour and repertoire structure the further study, especially on the wild population, is needed.

## **Influence of social type, context and sex**

### **Social type**

The significant influence of social type occurred among all repertoire signals in one to three parameters of each call type. The results showed that pair had significant influence on frequency parameter (Frequency 95% - in 95% of total energy) in twitter, which were highest when they expressed this vocalization type. Female, which were housed alone, expressed the loudest trill and contact song, and the Frequency range of duet was the lowest when the pair was in the enclosure with hatched chicks. These results may serve as a feature for monitoring the wild population and can lead to a more detailed description of their social group.

### **Context**

The influence of context was shown in trill, contact song and duet. From the previous characteristic of twitter when the individuals expressed it during all recording with no sign of distraction, is understandable that this signal was unaffected by context. In trill and contact song was shown that approach of keeper or me, and communication between individuals in contact song, significantly influenced the parameter of RMS amplitude (root-mean-square amp.). In both of these call categories the expression was loudest under these context condition. The context of approach had also an effect on duet in duration parameters that the duration was longer when keeper or me appeared. It seems according to these outcomes that the environment can influence the expression of vocal signals and therefore the environmental conditions should be taken into consideration in future research.

### **Sex**

The differences between sex was observed only in contact song in two frequency parameters (1st Quartile Frequency – in 25% of total energy, and Frequency 5% - in 5% of total energy) and one amplitude parameter (Max Time – first time with amplitude equal to

max amplitude) and these results showed that the females reach higher frequencies in the first 25% of the total vocalization energy. I suppose that in twitter are no significant differences between sex, because it is a short and simple call and it do not need to carry information about gender. I assume that trill may be an alert call (according to comparison with the vocalization of White-crested Laughingthrush) and for that reason there were no differences between sex.

The influence of sex initiated duet was also shown in few parameters (Aggregate Entropy – the disorder of sound, Bandwidth 90% - difference between 5% and 95% total energy of frequencies, Duration M1 – duration of the first part of male vocalization in duet, and Frequency 95% - in 95% of total energy). The outcomes showed that if the male initiated duet, its duration or first part of its duration was longer. In general, the parameters were significantly influenced if the male initiated duet.

## **Individual variability**

### **Differences between individuals**

In previous studies of individual recognition in many avian species and in songbirds as well have been observed that the frequency parameters may have greater potential to convey significant differences between individuals than the temporal or amplitude parameters (Bloomfield et al., 2005; Draganoiu et al., 2006; Vignal et al., 2007; Kennedy et al., 2009; Reers and Jacot, 2011). The same pattern occurs in the present research in the analysis of differences between individuals in the parameters of all four call types, mainly in twitter, trill and duet. Trill, contact song and duet vary between individuals in temporal and amplitude parameters as well, but not to such an extent as frequency. In twitter and trill the individual differences are influenced also by the rate of disorder (Aggregate entropy) in a sound probably because these call types are short calls and may be more sensitive to environmental conditions. The individual parameter (M elements – number of male's elements presented in duet) influence the individual distinctiveness of duet. The individuals show significant differences especially in trill parameters (in 43% of all variables), in twitter and duet is individual signature in 30% of all parameters and the lowest rate of disparities between individuals is in contact song where the differences were significant only in 9% from all parameters.

## Potential for Individuality Coding (PIC)

The key condition of individual identity and its recognition is that the variation within individuals is lower than among individuals. Despite the previous statement that the individuality is more often influenced by frequency parameters, it was not observed in the PIC of the call parameters, because the analysis of call types of Black-and-white Laughingthrush showed that most acoustic variables (all types) of each vocalization type may be used as cues for individual identity coding ( $PIC > 1$ ). In twitter the PIC value for all parameters (100%) was higher than one, in four parameters (duration, IQR Bandwidth - difference between the 1st and 3rd Quartile Frequencies, IQR Duration - difference between the 1st and 3rd Quartile Times, and RMS Amplitude - root-mean-square amp.) higher than two which indicates that these parameters may be the best indicators of individuality coding. In trill was also shown that all parameters (100%) may be used for individuality coding, but the parameter with the PIC value higher than two was just RMS Amplitude ( $PIC = 3,5$ ). Except one parameter all parameters (95,5%) may be used for individuality coding in contact song and three variables showed PIC value higher than two – M (male) elements, Frequency 5% (in 5% of total energy) and the best indicator for individuality coding F (female) trill cadency ( $PIC = 14,1$ ). Finally the duet showed PIC value higher than one in 22 from 32 duet parameters (69%) and in three were PIC higher than two - Duration M2 (duration of the second part of male vocalization in duet), Interval M1-M2 (between the first and second male's vocalization part in duet) and Start time male (according to female in duet), where is the most significant PIC value (12,6). Approximately 91% of all variables among the repertoire may be used for individuality coding.

These outcomes are comparable with results of other bird species where the variability between individuals is higher than variability within individuals, such as White-browed Warbler (*Basileuterus leucoblepharus*) (Aubin et al., 2004), Black-capped chickadee (*Poecile atricapillus*) (Christie et al., 2004), Carolina chickadee (*Poecile carolinensis*) (Bloomfield et al., 2005), Zebra finch (*Taeniopygia guttata castanotis*) (Vignal et al., 2007, Reers et al., 2011), Noisy miner (*Manorina melanocephala*) (Kennedy et al., 2009), Brownish-Flanked Bush Warbler (*Cettia fortipes*) (Xia et al., 2010 and 2012). These parameters with the highest potential of individuality coding need to be used in further study using playback experiment (Reers and Jacot, 2011).

## DFA

On the difference between individuals is participating mainly the frequency parameters. Although acoustic individuality can also be shown when taking a multivariate approach. The identification system should be based on all, frequency, time, amplitude and other parameters, that can reduce the risk of confusion (Mathevon, 1997; Aubin et al., 2007; Policht et al., 2009).

The DFA was performed only on duets, because the sample size of other call types was too small and for more detailed information about their individual discrimination is need to collect more data. Discrimination model included seven duet parameters three duration parameters (Duration Female, Duration of Twitter part M – male, F – female Trill Cadency), three frequency parameters (1st Quartile Frequency – in 25% of total energy, Frequency 5% - in 5% of total energy, Frequency 95% - in 95% of total energy) and one amplitude parameter (RMS amplitude - root-mean-square amp.). The result of probability of correct matching by chance was 20%, compared to the outcome of discriminant analysis, where the result was significantly higher with 75,8% success of correctly classified pairs. The outcome is comparable with results of other bird species, such as Spotted Antbird (*Hylophylax naevioides*) (73%; Bard et al., 2002), Rufous-and-white Wren and Niceforo's Wrens (80%; Valderrama et al., 2007), Brownish-flanked Bush Warbler (*Horornis fortipes*) ( more than 90%; Xia et al., 2012), Spanish sparrow (*Passer hispaniolensis*) (90,3%; Marques et al., 2004), Chiffchaff (*Phylloscopus collybita canarensis*) (98,2%; Naguib M, 2001).

In contrast, 11 captive mating pairs of Siberian cranes (*Grus leucogeranus*) duets (375 duets in total) were assigned to correct pair in 97,3% within three years (Bragina and Beme, 2010). Important condition for the individual identity recognition and correct matching of individuals is that the vocal parameters and differences between individuals in these parameters need to be stable over time (Ellis, 2008; Hoodless et al., 2008; Klenova et al., 2009; Xia et al., 2010; Odom et al., 2013). Given to this fact it would be necessary to obtain other data from shorter (e.g. weeks) and longer (e.g. several month, years) time period.

Previous studies on monomorphic bird species with parental care of both male and female showed that these species use acoustic clues for individual identification (Volodin, 2005; Klenova et al., 2009). According to results of the present research, it seems that



individual recognition should be important also for the Black-and-white Laughingthrush, which is monogamous and territorial species and both sex share parental care with both changing on the nest (personal communication with Stephan Bulk, 2013).

### **Monitoring and conservation**

Laughingthrushes are very difficult to observe, because they are shy species and they inhabit dense forest (Round, 2006). To study these and other similar species would be useful to combine acoustic technologies of monitoring and information about individual identity (Odom et al., 2013). The knowledge about individuality gathered from the acoustic analyses of bird vocalization is very valuable in many aspects of bird biology as population dynamics, behavioural ecology, sexual selection and territoriality. These information can bring new insights and may be used for classification, assessment and stabilization of taxonomic rank (Kumar, 2003; Odom et al., 2013). Volodin et al. (2005) also claim that the acoustical differences as a key for individual identity may improve the efficiency of acoustical recognition system as a tool for non-invasive monitoring in many species. However acoustic monitoring is not suitable for every species and environment. Terry et al. (2005) provided case studies on three different bird species (European bittern, Corncrake and Owl species) all with similar difficulties for observing, caused by the population size or distribution in dense habitat. The results showed that to be able to determine whether the individual or the vocalization is different, the vocalization may not be changed between larger time period (within years). And the places, where is a great overlap between birds vocalization and other noise occurs are not appropriate for acoustic monitoring as well (Bardeli et al., 2010). To determine whether the non-invasive monitoring will be suitable for Black-and-white Laughingthrush, further study on the stability of its individual identity is needed. The DFA on duets showed that this vocalization type may provide the ability to identify and discriminate individual pairs. It would be useful to test whether individual distinctiveness in duets would be stable over different time periods (weeks, month and years). It would be also convenient to collect more samples of each vocalization type to test individual differences in bigger extent, to determine whether birds really recognise individual differences which were found in this study.

For Black-and-white Laughingthrush is urgent further research on the wild population which will define the aspects of biology of this species and evaluate the

stability of the population (Shepherd, 2007). For example presenting the parameters of each call type which may encode individuality in the wild population and observing the responses of receivers (Tibbetts and Dale, 2007), or monitoring the individuals in reintroduction programs (Sutherland et al., 2010).

## 7 Conclusions

The study provided basic repertoire of Black-and-white Laughingthrush, which I determined from the data collected during the nesting season from 24 individuals. It includes four different types of vocalization: twitter, trill, contact song and duet. According to the outcomes, each of these vocal signals contains individual signature clues, which differ between individuals. The results showed that the variability among the individuals is higher than between individuals and thus the requirement for individual recognition was satisfied. The calculation of Potential for Individuality Coding using coefficients of variance between and among individuals revealed that all variables of twitter and trill, all except one parameter of contact song, and 22 from 32 duet variables may be used for individuality coding. Classification result of discriminant analysis showed 75,8% correct assignment of duets into the correct pair.

Further research would be suitable to test whether individual distinctiveness in duets and other vocalization types would be stable over different time periods and to find out whether individual identity in acoustic communication would be able to serve as a tool for non-invasive monitoring of Black-and-white Laughingthrush. In combination with other monitoring methods such as playback experiments, it could bring new insights into many aspects of the species life. It may re-estimate the population size and provide more detailed description about the behavioural ecology (e.g. about territorial behaviour, parent-offspring relationship and mating system). It can also provide useful control monitoring system of the species in reintroduction programs. The more information will be gathered, the more effective conservation efforts would be able to apply in the future protection of the species.

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