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Vocalizations of red-necked snow finch, *Pyrgilauda ruficollis* on the Tibetan Plateau, China – a syllable taxonomic signal?

Fu-Min LEI[1]*, Ai-Zhen WANG[1, 2], Gang WANG[2, 3] and Zuo-Hua YIN[1]

[1] *Institute of Zoology, Chinese Academy of Sciences, 25 Beisihuanxi Road, Haidian District, Beijing 100080, China; e-mail: leifm@ioz.ac.cn*

[2] *Graduate School of Chinese Academy of Sciences, Beijing 100039, China*

[3] *Current address: Department of Zoology, University of Washington, Seattle, WA 981951800, USA*

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A b s t r a c t . The paper reported vocalizations of endemic Tibetan Plateau steppe sparrow, red-necked snow finch (*Pyrgilauda ruficollis*) by using SAS-Lab Pro. The subject similarity contrast and multivariate contrast were used to study the complexity and stability of sounds. The results indicated that songs of *P. ruficollis* were highly complicated with varied song types, song phrases, song syllable and combination of them. One song syllable shared by snow finches (*Montifringilla nivalis henrici*, *M. adamsi*, *P. ruficollis* and *P. blanfordi*) was selected as indicator to compare the taxonomic relationship among them. The similarity contrast and cluster analysis through SPSS were conducted to construct the similarity tree based on this shared syllable's acoustic parameters including the highest frequency (HF), the lowest frequency (LF), the main frequency (MPF) and duration (DUR). The results revealed that the syllable similarities are accordant with taxonomic status suggested in previous studies based on morphologic, ecological and molecular analysis methods. The syllable shared by these snow finches were presumed to be homologous and derived from a common ancestry. Further studies on songs encoding phylogenetic signal of snow finches are needed.

Key words: *Pyrgilauda ruficollis*, song, homologous, taxonomic signal

Introduction

Bird vocalization plays an important role in systematics. Vocalization has been used in discovering new species, assessing the rank in closely related allopatric taxa as well as inferring relationships both within and between genera (A l s t r ö m 2001). The utility of acoustic characteristics in systematics depends on the recognition of shared derived homolgies (E l d r e d g e & C r a c r a f t 1980). Typically, call types, in which vocal learning is slight and species-specific characteristics are unimportant (as in short–range calls), are considered as the best material for systematics (M u n d i n g e r 1979). Therefore most studies on vocalization concerning the taxonomy and species limits are confined to non-songbirds or song birds with simple songs (M i l l e r 1996, P a y n e 1982, 1986, L e i 1999a, L e i & P a y n e et al. 2002). For example, M i l l e r (1996) construct a testable phylogeny and estimate their affinities based on similar series of calls of certain Calidridinae species for attracting mates that are considered homologues. M c C r a c k e n & S h e l d o n (1997) reported that the vocalization of grey heron (*Ardea cinerea*) show great significance for taxonomy and may reveal the phylogenetic information; they also suggested that some acoustic characteristics are induced by the syrinx structure and vocal behavior transmit through the long genetic history.

It seems more difficult when to utilize songs to infer taxonomic relationships. First, as far as vocalization is concerned, song organization and complexity are highly variable

* Corresponding author

among songbirds, some birds have only one single, simple and stereotyped song, while others have a repertoire of a few even up to several hundreds song types (Catchpole & Slater 1995, Hartshorne 1973). Second, many songbirds have the ability to learn songs even from other species, i.e. song can be culturally transmitted, which increases the difficulty to distinguish the homologous component from the learned. Additionally, due to the complication, diversification and biological significance, bird's repertoire, even the same song type or syllable sequence is always changeable in different season, so the first step for taxonomic approach is to obtain homologous song type, phrase or syllable (Miller 1996). And then, before selecting out the shared syllable in some birds with the related birds, it's necessary to study the song patterns in the species. Besides the taxonomic significance, the analysis of song pattern can facilitate further study in bird communication because birds are found to have the ability to recognize the similarities of song types (Horn & Falls 1988b), and also sensitive to the different combinations of song types (Horn 1987, Horn & Falls 1988a,1991). Using the presence or absence of 16 vocalization note types of songs, Helbig et al. (1996) construct the phylogeny in the Palearctic chiffchaff *Phylloscopus collybita* complex. But the songs of *Phylloscopus collybita* are some simple in term of organization. Is it possible to find some similar syllables or phrases shared by related bird species with more complex songs? Do quantitative characteristics of the presumed homologous really reflect the taxonomic relationship? The two questions will be addressed by focusing on red-necked snow finch (*Pyrgilauda ruficollis*) and its related species *Montifringilla nivalis henrici, M. adamsi* and *P. blanfordi*.

Red-necked snow finch is endemic to the Tibetan Plateau (Lei et al. 2003). There are five more closely related snow finch species nearly co-distributing at the similar high altitude habitats of the Tibetan Plateau facing the similar environments (Fu et al. 1998, Qu et al. 2004). Studies on vocalizations of snow finches have brought up debates regarding their taxonomic status (Gebauer & Kaiser 1994, Ivanitsky 1992). However, most studies on bird vocalizations in China focused on simple description of vocal behavior relating to spectrograms, fewer studies on vocalization consider the taxonomic significances (Lei 1999a,b).

On vocalization of red-necked snow finch, there are some documented word descriptions, e.g. call-note "düuid" and a magpie-like alarm-note (Ali & Ripley 1987), calls like "duuid" or "doooid", warning call like "jē, jē, jē" (Fu et al. 1998), and "duuid" (MacKinnon et al. 2000). Gebauer & Kaiser (1994) have reported vocal behavior of red-necked snow finch with sonograms in detail. All these simple descriptions and sonograms are basically useful for the further study of song complexity. However, up to date, more detailed data of the vocalization in red-necked snow finch and acoustic comparison are currently scarce. This study will examine the song complexity and diversity and other song characteristics of red-necked snow finch, which are considered as the key factors for species recognition, sexual selection, mating and breeding success (Weary et al. 1990, Wang et al. 2003), and try to assess if the acoustic similarities among four snow finches reflects the taxonomic relationship.

Material and Methods

Species for comparison included red-necked snow finch (*Pyrgilauda ruficollis*), plain-backed snow finch (*P. blanfordi*), snow finch (*M. nivalis henrici*) and black-winged snow finch (*M. adamsi*).

Song recordings

Songs were recorded from April to May 2000 during the early breeding season in Heimahe, Maduo and Huashixia in Qinghai Province, and from August to September 2001 in Bangda, Dingri, and Tuotuohe in Xizang Aut. Reg. Sounds were recorded with a Sony MD Walkman Digital Recorder and Minidisk recorder MZ-R50, using a Sondy ECR-598 directional microphone. Tapes used were Sony MDW-60 digital Audio MiniDisc.

Acoustic analysis software

Input of recordings to computer was 16 bit at a 22.05 kHz sampling rate. The vocalizations were analyzed with SAS-LAB PRO (Specht 1998). Sonograms were made with settings of 256 point transform and Hamming analysis window. The corresponding frequency resolution and bandth were 86 and 112Hz respectively. Overlap was set at 50% with the temporal resolution 5.8ms and 1/bandwidth 9.4ms.

Sonogram analysis

Kroodsma & Miller (1982) suggested that some birds appear to have the same basic call pattern throughout their sampled range, and others may have quite different patterns of the same call type in different regions. Variations can be detected in qualitative traits such as note form and syntax, and quantitative traits such as frequency and temporal parameters.

We analyzed the song type following Payne (2000), syllable and syntax following Balaban (1988). HF (the highest frequency), LF (the lowest frequency), MPF (the main frequency) and DUR (duration)were measured. For comparing the sound similarity, we used the subject similarity contrast (Payne et al. 1999) and multivariate contrast (Clark et al. 1987, Gaunt et al. 1994, Khanna et al. 1997). This method was successfully used in comparing some species with simple song and calls (Payne 1978, Trainer 1983, Shackell et al. 1988, Gaunt et al. 1994, Bell et al. 1998).

SAS-LAB PRO was used to analyze the vocalization of the Red-necked snow finch, and then compare some song parameters with other 3 snow finch species in both qualitative and quantitative traits.

Shared song syllable

Stereotyped songs or song units can be used in resolving taxonomic problem with phylogenetic meanings, e.g. cuckoos (Miller 1996, Payne 1997). In this study, we compared the similarities by song phrase and syllable shape between different individuals: the subject similarity was given a rank value: 0 means no similarity, 1 means slightly similar, 2 means similar and 3 means quite similar, and then the accumulated value of each phrase was then produced. The higher the value is, the higher the similarity becomes. From this high similar phrase the common shared syllable was selected to measure several parameters including HF, LF, MPF and DUR of this syllable and to set a database. Data were tested through multivariate contrast with software package of SPSS 10.0 for windows. Parameters with significant differences among species were used in clustering the relationship of all taxa on SPSS.

Results

Vocalization of the red – necked snow finch

Through sonogram analysis, no two completely song types were found. Sonograms show that songs are composed of much more diversified phrases and syllables. Some songs are long without a clear interval.

In the following 4 continuing clear songs selected (Fig. 1), a, b, c and d are all phrases from different areas, among which a has 7 main phrases; b has 14; c has 6; d has 11 phrases.

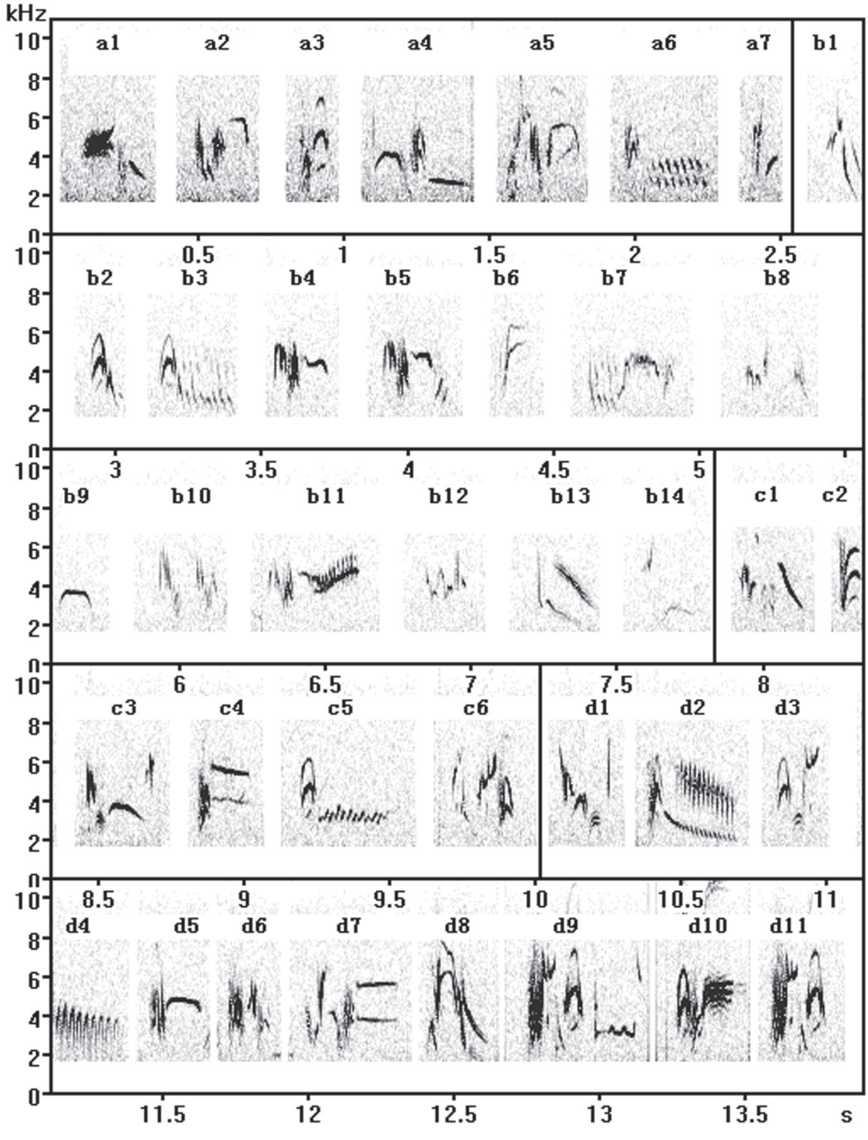

Fig. 1. Song motifs (song types) of different individuals of red-necked snow finch (recorded by L e i Fu-min at Heimahe, Qinghai from April to May 2000).

These phrases have been found with the following general characteristics:

1, The frequency range is from 2–6 kHz, duration is from 100–350ms, and each phrase consists of 1–3 syllables.

2, Within a song of an individual, the phrase is very highly stereotypical.

3, Within a song of an individual, a new phrase may occurred by adding or deleting certain syllable, e.g. b2 with b3; b4 with b5; d9 with d11 are all derived from the original b2, b4 and d9.

4, Within a song of an individual, phrases will last a long time before a new one occurs. Fig. 2 reflects the change orientation of phrase and clearly shows the stages pattern: including changeable stage (C), and relatively stable stage (A & B). In stage C, there are 3 sub-stages, the variation of song phrases are gradient.

5, Among different individuals, there are some similar syllables and phrases (with only one syllable). For example, in syllable a3, a6, b2, b3, c5, d3, d9 and d11, there is a common shared syllable with harmonics "X". Phrase c2 and b6 are similar. c4 and 2nd syllable of d7 are similar.

Similarity in the phrase level

There are 7–14 song phrases being found in red-necked snow finch songs. By using Payne's (1999) subject similarity contrast method to 8 individuals' songs (a, b, c, d, e, f, g, h), there are phrases a1-a7, b1-b14, c1-c6, d1-d11, e1-e9, f1-f5, g1-g6, h1-h4 respectively (Table 1). Table 1 lists the compared results of 4 phrases with the higher whole score.

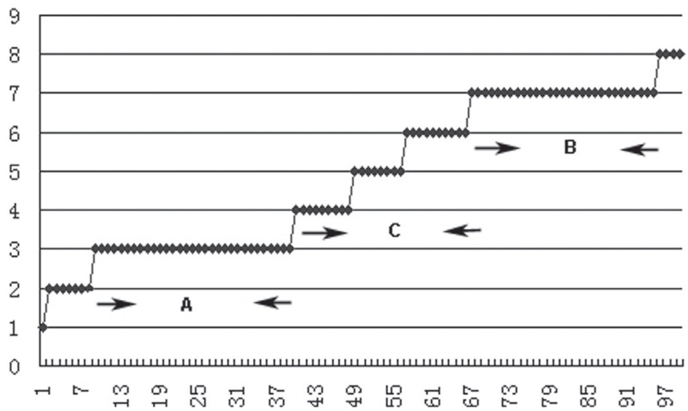

Fig. 2. The motif variation curve of one recorded song of a red-necked snow finch. The horizontal line represents the number of all of song motifs and the vertical line represents the number of different song motifs (recorded by Lei Fu-min at Heimahe, Qinghai in April 2000).

Table 1. Comparisons of song phrases' similarities.

Score rank	Phrase	Whole score
1	a6, b3, c5	10
2	a3, b2, d9, d11, e9	9
3	a7, b4, b6, c2, f3, f5	6
4	a4, c3, e7, g1	4

Similarity in the syllable level

Among similar phrases, there is always an identical syllable, "X" (Fig. 3). By comparing 8 individuals with this similar syllable, 10 phrases were selected (Fig. 4).

Measurements were taken from II of X syllable (Figs 3 and 5). By comparatively contrasting red-necked snow finch, plain-backed snow finch, snow finch and black-winged snow finch, the similar syllable commonly shared was selected (Fig. 6). We then measured 5 parameters of these snow finches, and used multivariate contrast through SPSS (Table 2). The results of paired t – test are listed in Table 3. As syllable X in the Table 3 was concerned, DUR of it in *P. ruficollis* is significantly different from that in other species, MPF is not significantly different from *P. blanfordi*, but significantly different from *M. nivalis henrici* and *M. adamsi*. HF and LF are not different from other species significantly.

We then selected DUR and MPF of the X syllable to cluster 10 individuals of *P. ruficollis*, 5 individuals of *P. blanfordi*, *M. adamsi* and *M. nivalis henrici* respectively. The clustering tree is as bellow (Fig. 7).

Fig. 8 showed that 10 individuals of *P. ruficollis* and 5 *P. blanfordi* clustered together. These two species were not distinctively separated. Different individuals of a species were almost clustered at a terminal branch except for *P. ruficollis* and *P. blanfordi*. This branch was then clustered with *M. adamsi* and *M. nivalis henrici* branch. Five individuals of *M. adamsi* are clustered at a terminal branch; five individuals of *M. nivalis henrici* are clustered at a terminal branch. Some individuals of *P. ruficollis* and *P. blanfordi* were not clustered at the terminal branch, but the spot scattering map shows the clear differences (Fig. 8).

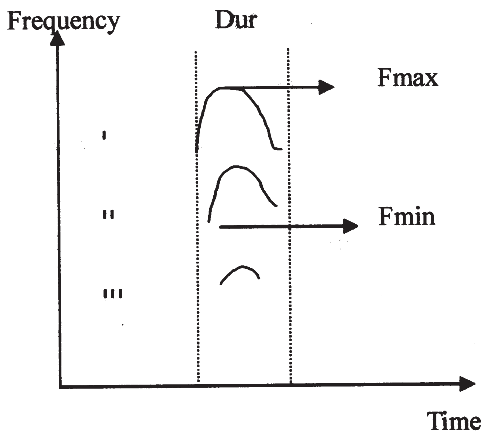

Fig. 3. The sketch figure of syllable X, which is made up of three parts: I, II & III.

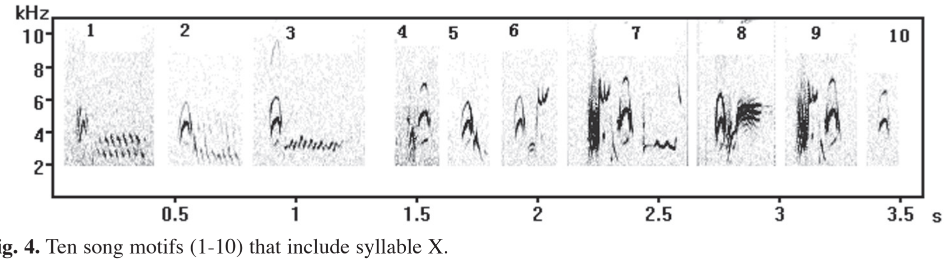

Fig. 4. Ten song motifs (1-10) that include syllable X.

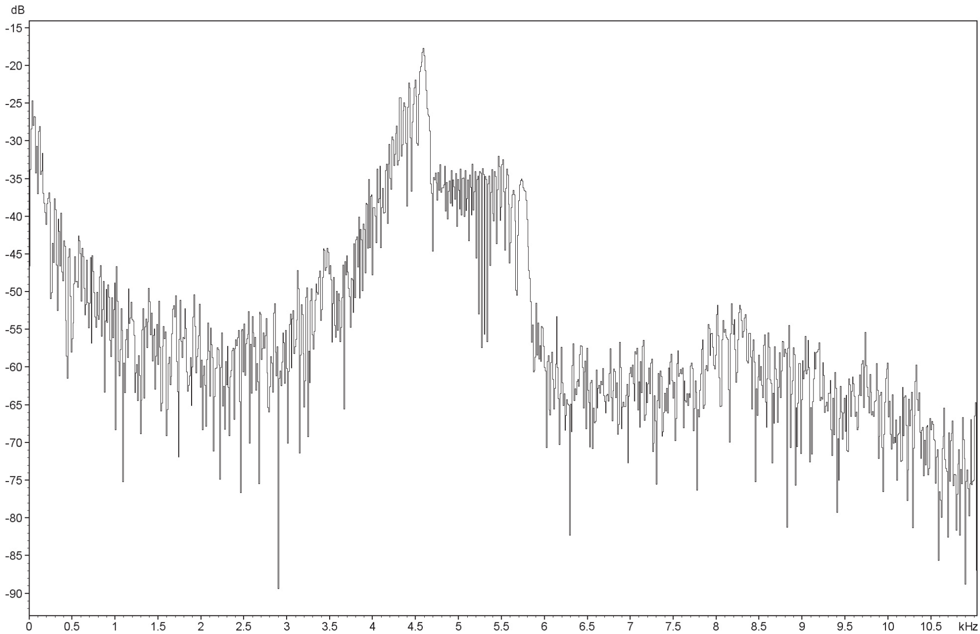

Fig. 5. The energy spectrum of a syllable X. The horizontal line represents the parameter of frequency, and the vertical line represents the parameter of energy.

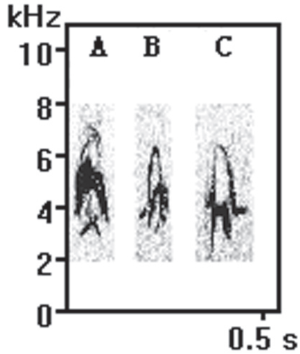

Fig. 6. The sonogram of syllable X (A - *M. adamsi*, B - *P. blanfordi*, C - *M. nivalis henrici*).

Table 2. Measurements of parameters of syllable X in *P. ruficollis*, *M. adamsi*, *P. blanfordi* and *M. nivalis henrici*.

	DUR(10^{-2}s)	MPF(10^{3}Hz)	FMAX(10^{3}Hz)	FMIN(10^{3}Hz)
P. ruficollis	5.85±0.66	4.79±0.32	6.56±0.58	3.66±0.34
M. adamsi	9.68±0.27	5.26±0.22	7.09±0.13	3.39±0.21
P. blanfordi	4.72±0.40	4.53±0.22	6.33±0.05	3.53±0.25
M. nivalis henrici	10.52±0.16	3.97±0.06	6.51±0.10	19.6±0.04

Table 3. The pair test of the parameters of syllable X of *P. ruficollis* with *M. adamsi*, *P. blanfordi*, *M. nivalis henrici* (P=0.05).

Acoustic parameters	Pair test	T value	Sig. (two-tailed)
DUR	*P. ruficollis—M. adamsi*	-16.506	.000
	P. ruficollis—P. blanfordi	3.710	.021
	P. ruficollis—M. nivalis henrici	-34.902	.000
MPF	*P. ruficollis—M. adamsi*	-5.489	.005
	P. ruficollis—P. blanfordi	1.319	.258
	P. ruficollis—M. nivalis henrici	6.362	.003
HF	*P. ruficollis—M. adamsi*	-2.464	.091
	P. ruficollis—P. blanfordi	-.237	.828
	P. ruficollis—M. nivalis henrici	-1.093	.354
LF	*P. ruficollis—M. adamsi*	1.234	.285
	P. ruficollis—P. blanfordi	.394	.714
	P. ruficollis—M. nivalis henrici	8.705	.001

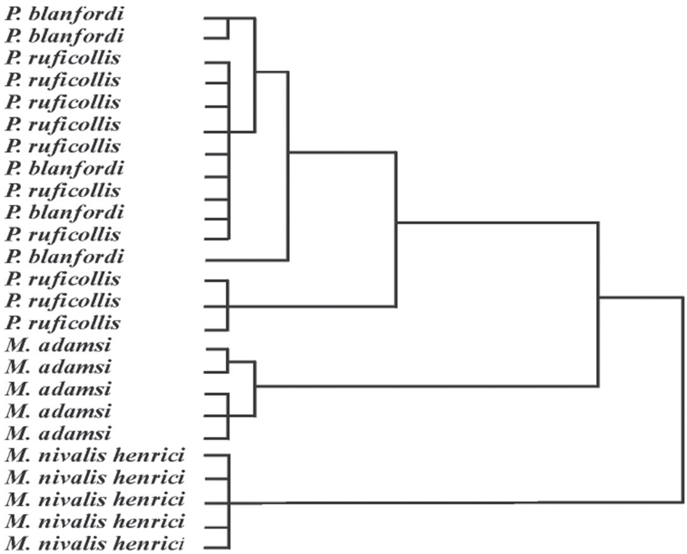

Fig. 7. The Average Cluster of *P. ruficollis*, *P. blanfordi*, *M. adamsi* and *M. nivalis henrici* by parameters of Duration (DUR) and the Frequency with the maximal amplitude (MPF) of the syllable X.

Discussion

Vocalizations and song complexity of red – necked snow finch

Bird vocalization is species-specific. It is very important for passerine birds in species recognition and thus co-distributed sympatric species breeding isolation, especially during the breeding season. Vocalization also functions in individual recognition in birds. Because of signaling males' status and other information, males' songs can be used by females to choose mates. Females in some bird species prefer to select males with large song repertoires as mates, so complex songs are often regarded as the results of sexual selection (Raymond 1999). Songs of red-necked snow finch were found complicated and diversified, especially

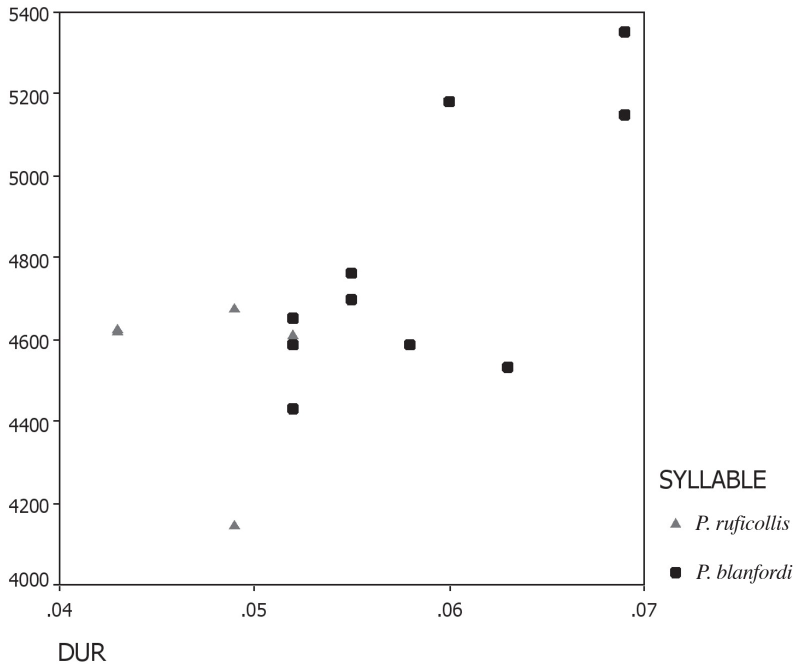

Fig. 8. The simple scatter plot of the syllable X of *P. ruficollis* and *P. blanfordi* by the parameters of DUR and MPF.

when the phrases were concerned. Some phrases were found highly repeated in the songs (Table 1), which might be related with the characteristics of strong wind, noise, high altitudes in the plateau habitats (3800–5000m). This repeat includes three different types: mono-phrases, bi-phrases, and poly-phrases repeat, or these mixed together. The variety results from different song types such as "immediate variety", "eventual variety" et al. as Verner (1975) introduced. Song type in red-necked snow finch is highly complicated taking on the model of "poly-syllable sequence unstable variety" as Lei et al. (2003) reviewed. The appearance of this so called "poly-syllable sequence unstable variety" indicates that bird song is very complicated with the strong female preference (Martens & Eck 1995, Payne 1986, 1997, Wang et al. 2003).

Because phrases in songs of red-necked snow finches are highly variable, it's hard to select a common shared phrase to process quantitative comparison analysis among the relative birds. But through the contrast of phrases among those species, we found a syllable named "X" being commonly shared. It is stable in red-necked snow finches and shared by any other species. By comparing the syllable among all 4 species of snow finches we selected (Table 4), t – test shows that the syllable is significantly different from other species in DUR and MPF, while the FMAX and FMIN are not significantly different among them. This indicates that bird may recognize individual "voice" with song features that are subtle and difficult to describe with simple sonograms (e.g. Cynx et al. 1990, Dhondt & Lambrechts 1992). FMAX and FMIN are clearly separated by spot scattering even though differences are not significant between species. Thus the syllable is commonly shared by the species complex in sonogram quality but species specific in parameters quantity. It might be homologous.

The homologous syllable – taxonomic signal?

Vocalizations of birds are very important signal for species taxonomy and phylogeny (reviewed in Payne 1986, Morel & Chappuis 1992, Lei & Wang 1999, and Lei et al. 2002). That song syllable is shared by these species indicate that it may derive from the common ancestry. Although normal bird songs develop under tutoring and learning, the songs still inherit species specific character. In several studied birds refuse to learn from other birds and young birds show the preference for songs of its own species in sensitive stage (Catchpole & Slater 1995, Wang et al. 2003). Thus songs' culture inheritance is under genetic control. Similarities in song quality may express genetic similarities even in which song is learned, and accordingly may be use in phylogenetic analyses (Payne 1986). And the difference between the similar characters may reflect the long divergence history.

In terms of the differences of general vocalization and ecological habitats, Gebauer & Kaiser (1994) and Ivanisky (1991) divided the snow finches into two different genera *Montifringilla* and *Pyrgilauda*. *M. nivalis* and *M. adamsi* were included in *Montifringilla*. Other five snow finches, *P. ruficollis*, *P. taczanowskii*, *P. blanfordi*, *P. davidiana* and *P. therease*, were in *Pyrgilauda*. The result of clustering in DUR and MPF suggests that *M. nivalis henrici* and *M. adamsi* are distinguished with *P. ruficollis* and *P. blanfordi*. Individuals of *Montifringilla* spp. are clustered together at the terminal branches. The acoustic similarity concluded in this study is generally accordant with taxonomic status of snow finches suggested by Gebauer & Kaiser (1994) and Ivanitsky (1991) based on behavior and habitats, suggested by Lei et al. (2001, 2003) based on numerical and cladistic classification, and suggested by Qu (2003) based on molecular methods. Although the parameters in DUR and MPF are not qualified to distinguish *P. ruficollis* and *P. blanfordi* from statistic t-test, but the spot scattering difference might be clear enough for the species recognition.

Qu et al (2004) further studied the systematic relationship among five species in *Pyrgiauda* based on morphologic character and molecular methods. They found the difference of *P. taczanowskii* between other four species is significant enough to be lifted as a genus *Onychostruthus*. More syllables from more individuals and species and more bioacoustic information are needed to compare with Qu's suggestion.

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