Organization and variation of the Tetraonidae (Aves: Galliformes) mitochondrial DNA control region

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ABSTRACT
The mitochondrial DNA control region is the most polymorphic region of the mitochondrial genome. To infer the organization and variation of Tetraonidae mitochondrial DNA control region, the entire control region sequences of 18 species were analyzed. The length of the control region sequences ranged from 1127 bp (Bonasa sewerzowi) to 1156 bp (Centrocercus minimus). The average genetic distances among the species within the genera varied from 0.53% (Tympanuchus) to 9.42% (Bonasa). The average genetic distances showed insignificantly negative correlation with ts/tv. Five conserved sequence boxes in the domain II of Tetraonidae sequences were identified. The alignment of the Tetraonidae five boxes and CSB-1 sequences showed a few sequence variations. The results indicated that the genus Dendragapus might not be monophyletic.

Tetraonidae is a group of birds from the order Galliformes, including six genera, eighteen species. Lucchini et al. (2001) preliminary described the structure of the mtDNA control region of Tetraonidae. But the rates and patterns of molecular evolution of Tetraonidae control region are not known. In the present study, we examined the organization and variation of the control region of Tetraonidae species retrieved from GenBank (Table 1). The aim is to characterize the structural features and patterns of sequence evolution of the Tetraonidae mitochondrial DNA control region.

A total of 18 species from 6 genera belonging to the Tetraonidae family were analyzed. All the Tetraonidae species had only one control region. The control region spans the region between the genes for tRNAGlu and tRNAPhe in the Tetraonidae species. The length of the control region is relatively conserved, about 1145 ± 11 bp, but that of Bonasa sewerzowi is only 1127 bp.

The average nucleotide composition of Tetraonidae control region sequences was as follows: 25.92%A, 33.65%T, 14.01%G and 26.43%C, with a bias against G. The amount of A + T was more than that of G + C among whole control region, especially in domain III, same as reported other avian control region (e.g. Marshall and Baker 1997; Ruokonen and Kvist 2002; Huang and Ke 2016).

Genetic distances between species ranged from 0.27% (between Tympanuchus phasianellus and Tympanuchus cupido) to 13.05% (between Tetrao urogallus and Bonasa sewerzowi), showing a wide range of divergences. The average genetic distances among the species within the genera varied from 0.53% (Tympanuchus) to 9.42% (Bonasa). The average genetic distances showed insignificantly negative correlation with ts/tv (r = −0.6852, p > .05).

Nucleotide substitutions occur more frequently in peripheral domains. Average substitution rate for the three domains was 0.51:0.22:0.27, corresponding to relative proportions of 5:2:3, respectively. Among all the genera of Tetraonidae, domain I is the most variable of the three domains (Figure 1).

Five conserved sequence boxes in the domain II of Tetraonidae sequences were localized and identified as boxes F, E, D, C and B. These boxes were also identified by Randi and Lucchini (1998) in Alectoris sequences, except B-box. In F-box, 21 of 28 nucleotide positions were fully conserved among the Tetraonidae sequences. There were both four nucleotide positions were variable in C-box and B-box, while there were only one and two nucleotide positions were variable in D-box and E-box, respectively.

On the basis of hierarchical likelihood ratio tests (hLRTs) as implemented in ModelTest 3.0, the model general time reversible (GTR) model + gamma distribution (G) + invariable (I) was used (GTR + G + I, −lnL = 4643.26, AIC = 9372.72, BIC = 9711.70). Maximum likelihood method was used to reconstruct the phylogenetic trees based on GTR + G + I model. Member of Tetraonidae was formed an alone clade, as a sister to out-group (Figure 2). Species of Bonasa was the first to split from the Tetraonidae lineage. All the genera could be discriminated by their distinct clades in the phylogenetic tree except Dendragapus. Our results indicated that the genus Dendragapus might not be monophyletic.
**Table 1** Species examined and source of sequence data in the present study.

| Genus      | Species                  | Length of the control region | Accession Number | Sources                        |
|------------|--------------------------|------------------------------|------------------|--------------------------------|
| Dendragapus| *Dendragapus falcipennis*| 1149                         | AJ297164         | Lucchini et al. 2001           |
|            | *Dendragapus canadensis* | 1147                         | AJ297163         | Lucchini et al. 2001           |
|            | *Dendragapus obscurus*   | 1150                         | AJ297161         | Lucchini et al. 2001           |
| Lagopus    | *Lagopus muta*           | 1142                         | AJ297170         | Lucchini et al. 2001           |
|            | *Lagopus leucura*        | 1143                         | AJ297168         | Lucchini et al. 2001           |
|            | *Tetrao tetrix*          | 1147                         | NC024554         | Li et al. 2016                  |
| Lagopus    | *Tetrao mlokosiewiczi*   | 1145                         | AJ297173         | Lucchini et al. 2001           |
|            | *Tetrao urogallus*       | 1146                         | DQ323553         | Alda et al. 2016, unp          |
|            | *Tetrao parvirostris*    | 1143                         | AJ297178         | Lucchini et al. 2001           |
| Bonasa     | *Bonasa sewerzowi*      | 1127                         | NC025318         | Li et al. 2014                  |
|            | *Bonasa umbellus*        | 1146                         | AJ297157         | Lucchini et al. 2001           |
|            | *Centrocercus urophasianus* | 1151                      | GQ902785         | Oyler-McCance et al. 2010, unp |
| Centrocercus| *Centrocercus minimus*   | 1156                         | GQ902778         | Oyler-McCance et al. 2010, unp |
|            | *Tympanuchus phasianellus* | 1148               | AJ297176         | Lucchini et al. 2001           |
| Tympanuchus| *Tympanuchus cupido*     | 1148                         | AJ297171         | Lucchini et al. 2001           |
|            | *Tympanuchus pallidicinctus* | 1147             | AJ297174         | Lucchini et al. 2001           |
|            | *Bambusicola thoracica*  | 1146                         | EU165706         | Shen et al. 2010               |
| Outgroup   | *Bambusicola fytchii*    | 1174                         | NC020583         | Shen et al. 2010               |

**Figure 1.** Distribution of the variable sites in the control region. The number of variable sites within genera has been plotted in 50-bp intervals.
Disclosure statement

The authors report no conflicts of interest.

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Figure 2. Phylogenetic tree of Tetraonidae constructed from mitochondrial DNA control region sequence. Numbers at nodes indicate bootstrap values (≥80%) from 1000 replications.

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