Relationships of *Syrmaticus* and *Phasianus* by Cyt-*b* Gene Array Comparison

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The relationships of *Syrmaticus* to *Phasianus* remain uncertain and opinions differ whether they are separate genera or congeneric. We examined the relationships of *Syrmaticus* to *Phasianus*, *Lophura*, and *Catreus* by means of Cyt-*b* gene array comparisons. As a result, we found that each of the following groups forms a cluster, (1) *Syrmaticus soemmerringii*, (2) *Syrmaticus reevesii*, (3) *Syrmaticus ellioti*, *S. humiae*, *S. mikado* and (4) *Lophura erythrophthalma*, *Catreus wallichii* and *Phasianus versicolor*. There was 10.81% difference between cluster(3), Elliot's, Hume's and Mikado Pheasant, and cluster(4), Crestless Fireback, Cheer and Green Pheasant (*Phasiunus*). The Cheer Pheasant, the taxonomic position of which has been debated, was included in the Green Pheasant cluster in our experimental data and hence considered to be more closely related to *Phasianus* than to *Syrmaticus*.

Key Words: Classification, Cyt-*b* gene, *Phasianus*, *Syrmaticus*

There are various opinions regarding the classification of the Copper Pheasant. Delacour (1977) and Johnsgard (1988) claim that the Copper Pheasant is of the genus *Syrmaticus* and includes five species, namely, Reeve's Pheasant, Mrs. Hume's Pheasant, Elliot's Pheasant, Mikado Pheasant and Copper Pheasant. However, Peters (1934), while recognizing the genus *Syrmaticus*, divides it into four subgenera, which he refers to as *Syrmaticus*, *Graphephasianus*, *Calophasis* and *Cyanophasis*. Checklist of Japanese Birds (The Ornithological Society of Japan 1974) includes these in the genus *Phasianus* and does not recognize the genus *Syrmaticus*. As reasons for recognizing the genus *Syrmaticus*, Delacour (1977) points out the clear differences between the down of the chicks in the two genera and that the five species of the genus *Syrmaticus* form a natural group clearly distinct from the genus *Phasianus*. Johnsgard(1986) claims that the genera *Phasianus* and *Syrmaticus* are not greatly divergent in terms of evolutionary history.

Morioka(1988), in common with Delacour (1977) and Johnsgard (1988), also considers the genus *Phasianus* and the genus *Syrmaticus* to be relatively close within the sub-family Phasianinae. However, since the genus *Syrmaticus* is sufficiently distinct in form and physiology, it should be considered an independent genus.

In terms of form, though the significance is not clear, Reeve's Pheasant has the

most narrow facial protrusion and the most highly developed tail feathers of all of the species in the genus *Syrnaticus*. In addition, it also has the most extensive white portion on the head, an important signal of the male, and, in these respects, the Reeve's Pheasant differs from the other four species of Long-tailed Pheasants (*Syrnaticus*).

It is well known that the fertility of the F1 generation due to crossbreeding is an important tool in understanding affinity relationships and a number of examples have been reported.

Although the Reeve's Pheasant will crossbreed with the Copper Pheasant, the F1 generation has low fertility (Delacour, 1977) and, when crossbred with the Ring-necked Pheasant, F1 females are usually sterile and males have a low level of fertility (Knoder, 1963). These examples indicate that these two groups are not closely related.

However, since the genus *Syrnaticus* remains in doubt not only in its relationships to the genus *Phasianus* but also in terms of its taxonomic positioning within the genus, we attempted to clarify the unknowns in terms of molecular biology from mitochondrial *Cyt-b* gene arrays.

MATERIALS AND METHODS

In this research, the Northern Copper Pheasant (*Syrnaticus soemmerringii scintillans*), Elliot's Pheasant (*Syrnaticus ellioti*), Hume's Pheasant (*Syrnaticus humiae*), Mikado Pheasant (*Syrnaticus mikado*), Reeve's Pheasant (*Syrnaticus reevesii*), Crestless Fireback Pheasant (*Lophura erythrophthalma*), Cheer Pheasant (*Catreus wallichii*) and Green Pheasant (*Phasianus versicolor*) were used and the Red Jungle Fowl *Gallus gallus* was used as the out group.

DNA samples were extracted from blood samples or from livers taken from dead specimens using the usual methods.

DNA amplification was carried out by the PCR method and the primers used were the AGCCCATCCAACATCTCTGCTTGATGAAA and TGGGGCCAAATATCATTCTGAGGGGCCACC arrays of Irwin *et al.* (1991) shortened to 30bp with positioning at 14961-15327 (refer to Desjardins & Morais 1990). The composition of the PCR reactant was 10 μ l of buffer used for *Taq* DNA polymerase and *Taq* DNA polymerase 2U (TAKARA), 20 pmol of each primer, 200 μ M of each dNTPs and the total amount was adjusted to 100 μ l each with distilled water. DNA used as the template was diluted step by step with sterilized distilled water and used after confirming the optimal concentration.

A Perkin Elmer Cetus PJ-200 thermal cycler was used in the PCR and we repeated the cycle of 94°C for 1 minute, 58°C for 1 minute and 72°C for 2 minutes 35 times after preheating to 94°C for 1 minute. After the completion of the reaction, samples were extracted from each sample tube and the outcome of the PCR was confirmed by means of agarose cataphoresis.

Since large quantities of unreacted dNTPs and oligomerized primer were included in the PCR reactant, refinement was carried out using the agarose purification method prior to sequencing. We carried out the dye terminator cycle sequencing reaction with 3 pmol of sequencing primer and about 0.1 μ g of the refined PCR product as the template and determined the arrays using an auto-sequencer (ABI model 373A-36). Unreadable primers, misread bases and bases impossible to

analyze were determined through comparisons to the complementary base pair. In regard to the decoded 267bp, sequence divergence was computed using the computer program Phylip 3.4 and the phylogenetic tree was formulated through NJ using the computer program DNASIS. Though there are many opinions regarding scientific name, we followed Delacour (1977).

RESULTS

In order to confirm the taxonomic positioning of the genus *Syrmaticus*, we decoded *Cyt-b* gene 267bp arrays of species classified as belonging to the genera *Lophura*, *Catreus* and *Phasianus* and carried out comparisons. The results are as indicated in Tables 1, 2 and Fig. 1.

Fig. 1. A phylogenetic tree for the *Cyt-b* gene constructed by the NJ method. Numbers above each node represent the bootstrap support (of 1000 replicates) for each branch.

Remarks: Ss: *Syrmaticus soemmerringii scintillans*, Sr: *Syrmaticus reevesii*, Sm: *Syrmaticus mikado*, Se: *Syrmaticus ellioti*, Sh: *Syrmaticus humiae*, Cw: *Catreus wallichii*, Le: *Lophura erythrophthalma*, Pv: *Phasianus versicolor*, GgT: *Gallus gallus* (out group) numbered bootstrap

On the basis of the extent of sequence divergence (Table 2), we constructed the phylogenetic tree for the *Cyt-b* gene of the Long-tailed Pheasant group and the group containing some true Pheasants by NJ method (Fig. 1).

The *Cyt-b* gene tree indicated that the Copper Pheasant (*Syrmaticus soemmerringii scintillans*) diverged the earliest from the others, Reeve's Pheasant then diverged, and the other 3 species of the Long-tailed Pheasant group and Green Pheasant group diverged, after the Green Pheasant group diverged from the Long-tailed Pheasant group. Between the Green Pheasant (*Phasianus*) and the group containing some of the Long-tailed Pheasants (Elliot's, Hume and Mikado Pheasants) of the genus *Syrmaticus*, the rate of SD ranged from 7.87% (Elliot's Pheasant) to 10.81% (Mikado Pheasant) .

The time of divergence between clusters (3) and (4) is calculated to be 5.70 Myr.

Table 1. *Cyt-b* gene sequences of Long-tailed pheasants and related species

	10	20	30	40	50	60	70	80	90
<i>S.s.scintillans</i>	CTCACGGGC	TACTACTAGC	CATACATTAC	ACCGAGATA	CCTCCTGGC	CTTCTCTCC	GTAGCCACA	CATGTGAAA	CGTACAATAC
<i>S.elliotti</i>	-----	-----	-----	-T-----C-	-----A-	-----	-----	-C-----	-----
<i>S.humiae</i>	-----	-----	-----	-T-----C-	-----A-	-----	-----	-C-----	-----
<i>S.mikado</i>	-----	-----	-----	-T-----C-	-----A-	-----	-----	-C-----	-T-----
<i>S.reevesii</i>	-----	-T-----	-----	-T-----C-	-----A-	-T-----	-G-----	-T-C-----	-----
<i>L.nycthemera</i>	-----	-C-C-----	A-----	-C-----	-A-----T-	-A-----	-T-----	-----	-T-----
<i>C.wallichii</i>	-----	-C-----	-C-----	-----	-A-----	-----	-----	-----	-----
<i>P.versicolor</i>	-----	-C-----T-G-	-----	-----	-----	-----	-----	-----	-----
<i>Gallus gallus</i>	CTCACGGGC	TACTACTAGC	CATGCACTAC	ACAGAGACA	CATCCTAGC	CTTCTCTCC	GTAGCCACA	CTTGCCGAA	CGTACAATAT
<i>S.s.scintillans</i>	110	120	130	140	150	160	170	180	190
<i>S.elliotti</i>	TCGAAACCT	CCATGCAAT	GGGGCTCAC	TCTTCTCAT	CTGCATCTTC	CTTCACATCG	GCGAGGCGT	ATACTACGGC	TCCTACCTAT
<i>S.humiae</i>	-----T-	-----C	-----	-T-----	-T-----	-----	-C-----	G-----	-T-----
<i>S.mikado</i>	-----T-	-----C	-----	-T-----	-T-----	-----	-C-----	G-----	-T-----
<i>S.reevesii</i>	-----T-	-----C	-----	-T-----	-T-----	-----	-C-----T-	G-----	-A-----
<i>L.erythrophthalmus</i>	-----	-----	-----	-T-----	-T-----	-----	-C-----	-A-----	-G-----
<i>C.wallichii</i>	-----T-	A-----	-----	-T-----	-----	-C-----	-A-C-----	C-----	-----
<i>P.versicolor</i>	-----T-	-----	-----	-T-----	-----	-T-----	-A-C-----	T-----	-T-----
<i>Gallus gallus</i>	TCGGAAATCT	CCACGCAAC	GGGGCTCAT	TCTTCTCAT	CTGTATCTTC	CTCCACATCG	GACGAGGCGT	ATACTACGGC	TCCTACCTCT
<i>S.s.scintillans</i>	210	220	230	240	250	260	267		
<i>S.elliotti</i>	ATGAAACACC	GGTGTCATTC	TCCTCTCCAC	ACTCATAGCA	ACGGGCTTGG	TAGGATATCT	TCCTCCA		
<i>S.humiae</i>	-----	-A-----T	-A-----	-----	-----	-----	C-----T-		
<i>S.mikado</i>	-----	-A-----T	-A-----	-----	-----	-----	C-----T-		
<i>S.reevesii</i>	-----	-A-----T	-A-----	-----	-----	-G-C-----	C-----		
<i>L.erythrophthalma</i>	-----T-T	-A-----T	-A-----T-	-T-----	-----	-----	C-----		
<i>C.wallichii</i>	-----	-A-----T	-A-----	-----	-----	-C-----	C-----		
<i>P.versicolor</i>	-----	-A-----CT	-A-----T-	-----	-----	-C-----	C-----T-		
<i>Gallus gallus</i>	-----T	-A-----	-A-----	-----	-----	-----	C-----T-		
	CTGAAACACA	GGAGTAATCC	TCCTCTCCAC	ACTCATAGCC	ACGGGCTTGG	TGGGCTATCT	TCCTCCA		

Table 2. Sequence divergence of genus *Syrmaticus* and some related species.

Species	Sequence divergence(%)								
	0	1	2	3	4	5	6	7	8
0. <i>Gallus gallus</i>	—	12.35	14.16	13.71	15.56	16.53	15.15	15.61	16.02
1. <i>Syrmaticus s.scintillans</i>	26/241*	—	7.46	7.87	9.13	9.99	7.95	9.20	10.44
2. <i>Syrmaticus ellioti</i>	34/233	9/248	---	0.38	2.67	9.92	8.71	7.48	7.87
3. <i>Syrmaticus humiae</i>	33/234	20/247	1/266	---	3.05	10.34	9.13	7.89	8.28
4. <i>Syrmaticus mikado</i>	37/230	22/245	7/260	8/259	---	12.00	9.57	8.32	10.81
5. <i>Syrmaticus reevesii</i>	40/227	21/246	22/245	25/242	29/238	---	11.72	10.44	11.27
6. <i>Lophura erythrophthalma</i>	36/231	18/249	24/243	25/242	26/241	27/240	---	6.63	9.47
7. <i>Catreus wallichii</i>	38/229	21/246	20/247	21/246	22/245	24/243	22/245	---	7.83
8. <i>Phasianus versicolor</i>	37/230	22/245	19/248	21/246	28/239	25/242	26/241	24/243	---

*Number of base pairs(different/common).

to 1.96 Myr. using evolution speed of 2-4%/Myr. of Wilson *et al.* (1985).

In comparisons to species in other genera, the Cheer Pheasant at 7.83% had the closest relationships among the Green Pheasants. In addition, among the Copper Pheasants, the Crestless Fireback Pheasant of genus *Lophura* was 9.20% and the Green Pheasant of the genus *Phasianus* was 10.44%.

DISCUSSION

When we examined the genera *Catreus*, *Phasianus* and *Lophura*, which are considered to be closely allied to genus *Syrmaticus*, they were divided between the cluster containing the genus *Phasianus* and the various clusters containing the genera classified as the genus *Syrmaticus*. The Copper Pheasant included in the genus *Syrmaticus* and the four other Long-tailed Pheasant species were recognized to have a disparity of 7.46-10.44%.

There are two theories regarding the origin of the genus *Phasianus*, namely, the Common Pheasant type either from the west or from southern China. Of these, Kuroda & Ito (1987) consider the latter to be the most promising and point out the need to survey the Common Pheasant (*Phasianus colchicus*) group. Based on the theory of the origin from southern China, a close relationship to the genus *Syrmaticus* would not be strange since it is considered to have originated in the same region.

Delacour (1977) and Morioka (1988) claim that the genus *Phasianus* and *Syrmaticus* should be separated due to differences in form. At the present stage, the difference between (3) cluster, including the Elliot, Hume and Mikado Pheasant, and the Green Pheasant is recognized to be 10.81%.

Johnsgard (1986) and Kuroda & Ito (1987) position it as a sub-genus group; however, it is obvious that it is moving toward separation and, therefore, there would probably be no problem in isolating this as a separate genus. If we must have 2 separate genera, then we should use the genus name *Syrmaticus* because

Syrmaticus is positioned at the root of this tree.

The Cheer Pheasant is unique in being considered the sole species of the genus and there are various theories regarding its classification. Comparisons of *Cyt-b* gene arrays indicate that it is included in the same cluster as the genus *Phasianus*, which coincides with the results of research by Morioka (1988), who claims that it is closely related to the genus *Phasianus*. Although it is currently positioned before the genus *Syrmaticus*, it would probably be reasonable to place it between the two genera *Syrmaticus* and *Phasianus*.

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ヤマドリ属の分類学的関係

(Relationships of *Syrmaticus* and *Phasianus* by
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西5丁目

ヤマドリ属とキジ属の分類は定かではなく、1つの属とする説と分離独立させるとする2つの説があり、いまだ論争が続いている。ヤマドリ属 (genus *Syrmaticus*) の分類学的位置づけを確定するため、キジ属 (genus *Phasianus*) および両属に近いとされるコシアカキジ属 (genus *Lophura*)、カンムリキジ属 (genus *Catreus*) の類縁関係について *Cyt-b* 遺伝子配列の比較から検討をおこなった。

1) ヤマドリと2) オナガキジと3) カラヤマドリ・ビルマカラヤマドリ・ミカドキジと4) ウチワキジ・エボシキジ・ニホンキジがそれぞれクラスターを形成した。分類学的位置づけが論議されているエボシキジ (Cheer Pheasant) はニホンキジ (Green Pheasant) のクラスターに含まれ、ヤマドリ属 (genus *Syrmaticus*) よりもむしろキジ属 (genus *Phasianus*) に近かった。