## The genetic link between the Chinese bamboo partridge (*Bambusicola thoracica*) and the chicken and junglefowls of the genus *Gallus*

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Further comparison of mitochondrial con-ABSTRACT trol-region DNA base sequences of 16 avian species belonging to the subfamily Phasianinae revealed the following: (i) Generalized perdicine birds (quails and partridges) are of ancient lineages. Even the closest pair, the common quail (Coturnix coturnix japonica) and the Chinese bamboo partridge (Bambusicola thoracica), maintained only 85.71% identity. (ii) The 12 species of phasianine birds previously and presently studied belonged to three distinct branches. The first branch was made exclusively of members of the genus Gallus, while the second branch was made of pheasants of the genera Phasianus, Chrysolophus, and Syrmaticus. Gallopheasants of the genus Lophura were distant cousins to these pheasants. The great argus (Argusianus argus) and peafowls of the genus Pavo constituted the third branch. The position of peacock-pheasants of the genus *Polyplectron* in the third branch was similar to that of the genus Lophura in the second branch. Members of the fourth phasianine branch, such as tragopans and monals, were not included in the present study. (iii) The one perdicine species, Bambusicola thoracica, was more closely related to phasianine genera Gallus and Pavo than to members of other perdicine genera. The above might indicate that Bambusicola belong to one-stem perdicine lineage that later splits into two sublineages that yielded phasianine birds, one evolving to Gallus, and the other differentiating toward Pavo and its allies.

In the previous paper (1), we established duplication of the 60-base-long unit within the mitochondrial control (D-loop) region to be the characteristic unique to the genus *Gallus* among the phasianine birds. Furthermore, base sequence comparison of this maternally derived noncoding region revealed that as diverse as domestic breeds of chicken are, they could only have been derived from continental subspecies (e.g., *Gallus gallus gallus, Gallus gallus spadiceus*) but not an island subspecies (*Gallus gallus bankiva*) of the red junglefowl (*Gallus gallus*) (1). Excluded from the ancestry of domesticated chicken were three other species of junglefowls: the green (*Gallus varius*), the grey (*Gallus sonneratii*), and the Sri Lanka (*Gallus lafayettei*).

In the present study, we wish to define the position of the genus *Gallus* within the subfamily *Phasianinae* in relation to other members. The present study did not deal with members of the four other subfamilies that together with *Phasianinae* constitute the family *Phasianidae*. They were turkeys of the New World subfamily *Meleagridinae*, guinea fowls of the African subfamily *Numidinae*, and toothed quails and partridges of the New World subfamily *Odontophorinae*. Grouses of the Old World subfamily *Tetraoninae* were also excluded.

Because of their ornamental values, large pheasant-like birds of the subfamily *Phasianinae* are widely kept and propagated in various zoos of the world, as well as by private fanciers. This is not the case with diverse generalized perdicine birds (quails and partridges) of the same subfamily. If a few of them are kept and propagated, they are maintained only as exotic game birds. Accordingly, we were able to secure blood samples from only five species, although from four (five) different genera, of the generalized perdicine birds, which are said to consist of 103 species belonging to 22 different genera (2). They were the common quail of Japanese variety, Coturnix coturnix japonica, the blue-breasted quail (Coturnix sinesis, also known as Excalifactoria sinensis), the Chinese bamboo partridge Bambusicola thoracica, the chukar partridge, Alectoris graeca chukar, and the European grey partridge Perdix perdix perdix. In sharp contrast, there were no difficulties in securing 12 species of the large pheasant-like birds of the subfamily Phasianinae, representing 8 different genera; thus 11 of the 49 extant species were sampled (2). In addition to the red (G. gallus) and green (G. varius) previously reported (1), the genus Gallus was represented by two additional junglefowl species, the grey (G. sonneratii) and Sri Lanka (G. lafayettei) junglefowls. Pheasants, as such, were represented by three species: the ringnecked (Phasianus colchicus), the golden (Chrysolophus pictus), and Mrs. Hume's (Symmaticus huminae). Other large bodied birds of this subfamily that were also called pheasants, for the want of more descriptive words, were the silver gallopheasant (Lophura nycthemera), the Burmese peacockpheasant (Polyplectron bicalcaratum), and the great argus pheasant (Argusianus argus), which is as large as a peafowl. The peafowl of the genus Pavo is represented by the common peafowl (Pavo cristatus) and the green peafowl (Pavo muticus). Unfortunately, we were unable to secure samples of one important group of phasianine birds represented by tragopans, monals, and others.

As before, DNA were extracted from blood samples obtained from live birds with no apparent harm to them. Amplified copies of the mitochondrial control region were obtained by PCR reaction using two described primers (1). The same DNA-sequencing procedure as before was used (1). As a rule, at least two individuals of each species were analyzed.<sup>¶</sup>

Dendrograms were constructed on the basis of the neighborjoining method (3), which is based upon the result of a formal mathematical analysis (4) of Kimura's six-parameter model for computation of nucleotide substitutions (5).

## **OBSERVATIONS**

Sequences at the 392 positions of the mitochondrial control region of 14 species not given in our previous paper (1) are aligned and shown in three parts in Figs. 1 and 2. As shown previously (1), the 60-base-long unit containing the nearly

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<sup>&</sup>lt;sup>¶</sup>The sequences reported in this paper have been deposited in the GenBank data base (accession nos. D64163, D64164, D66888–D66900).

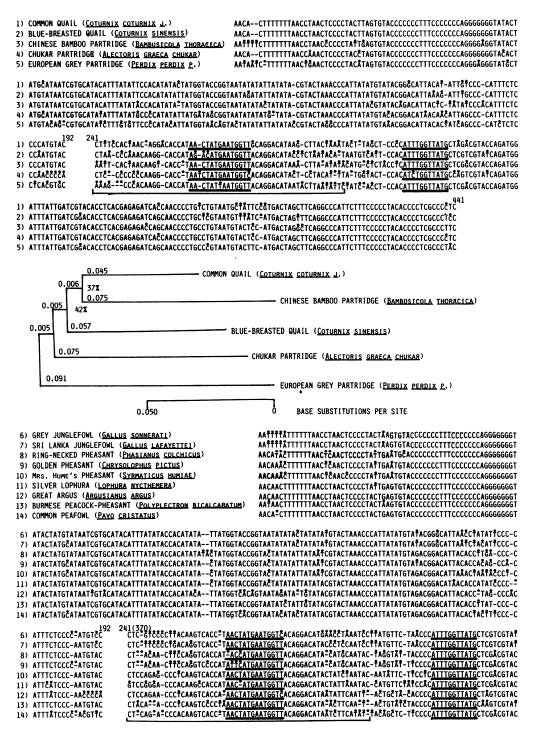


FIG. 1. (Top) The 392 positions (nt 1–192 and nt 241–441) of the mitochondrial control-region base sequences of the five perdicine species are aligned and shown in four rows. The consensus base of each position is shown with a large capital letter, while other bases are shown in small capital letters adorned with asterisks. The largely invariant tetradecameric core of the 60-base-long unit is underlined with a solid bar, as is the subsequent, nearly equally invariant monodecameric unit. The length of the 60-base long unit is also indicated by thin underlining. (*Middle*) On the basis of the above sequence comparison, the dendrogram of five perdicine species is drawn in accordance with the neighbor-joining method (3). Only shared sites were dealt with, and the length of each line indicates the distance from a branch point expressed as the number of base substitutions per site. The bootstrap probability of each estimated distance being correct is expressed as a percentage at each branch point based upon 1000 replication trials. (*Bottom*) Twelve phasiane mitochondrial sequences are aligned (and continued on Fig. 2 Top).

invariant tetradecamer AACTATGAATGGTT in its center is duplicated only in members of the genus *Gallus* and in no other. Accordingly, our interspecific sequence comparison only involved the original of this 60-base-long unit. Further, as also shown previously, the roughly 50-base-long region immediately in front of this 60-base-long region or its duplicated copy is hypervariable in all species (1). Because liberal introduction of gaps was needed to maximize interspecific homology of this hypervariable region, it was thought advisable to eliminate this region from the present sequence comparison. Accordingly, the dendrogram of perdicine and phasianine birds shown in Figs. 1 and 2 was drawn based upon differences at a total of 392 positions: positions 1–192 and then positions 241–441 (307–507 or 370–570 in the case of *Gallus*, depending

6) CAGATGGATTTATTGATCGT<sup>2</sup>CACCTCACGAGAGATCAGCAACCCCCTGCCCTGTAATGTACTCATGAC2AG<sup>2</sup>CTCAGGCCCATTCTTTCCCCCCTACACCCCTGCCCT<sup>2</sup>CT<sup>2</sup> 2) CAGATGGATTTATTGATCGT<sup>2</sup>CACCTCACGAGAGATCAGCAACCCCCTGCCCTGCCCTGTAATGTACTCATGAC2AG<sup>2</sup>CTCAGGCCCATTCTTTCCCCCCTACACCCCTGCCCT<sup>2</sup>CTC<sup>2</sup> 7) CAGATGGATTTATTGATCGT#CACCTCACGAGAGATCAGCAACCCCTGCCTGTCATGTACTTCATGAC#AG#ETCAGGCCCATTCTTTCCCCCCTACCCCCTCGCCCT#CTTG 8) CAGATGGATTTATTGATCGTACGCCCCACGAGAGATCAÈCAACCCCTGCCTÄTAATGTACTÈCATGACTAGÈTTCAGGCCCATTCTTTCCCCCCTACACCCCCTCGCCCTÄCATG 10) CAGATGGATTTATTÄATCGTACACCTCACGAGAGATCAČCAACCCCTGTCTATATGTATTČC&TGACŽAGÄČTCAGGCCCATTCTTTCCCCCCTACACCCCTCGČCCČTCTTG 10) CABATGGATTTATTGATCGTACACCTCACGAGAGATCAGCAACCCCTGCC<sup>2</sup>GTAATGTACTTCATGACTAGGTTCAGGCCCATTCTTTCCCCCTACACCCCCTGC 12) CAGATGGATTTATTGATCGTACACCTCACGAGAGATCAGCAACCCCTGCC<sup>2</sup>GTAATGTACTTCATGACTAGGTTCAGGCCCATTCTTTCCCCCTT 13) CAGATGGATTTATTGATCG&ACACCTCACGAGAGATCAGCAACCCCTGCC\*GTAATGTAC&&#ATGACTAGGTTCAGGCCCATTCTTTCCCCCTACACCCCTCGCCCT&CTTG 0.020 - GREEN JUNGLEFOWL (GALLUS YARIUS) 0.044 RED JUNGLEFONL (GALLUS GALLUS) 99.92 008 0.022 GREY JUNGLEFOWL (GALLUS SONNERATLI) 46. 0.01 0.015 SRI LANKA JUNGLEFOWL (GALLUS LAFAYETTEL) 0.005 0.019 RING-NECK PHEASANT (PHASIANUS COLCHICUS) 0.027 98.33 0.025 GOLDEN PHEASANT (CHRYSOLOPHUS PICTUS) 96.71 0.041 0.010 MRS, HUME'S PHEASANT (SYRMATICUS HUMLAE) 63.55 0.039 SILVER LOPHURA (LOPHURA NYCTHEMERA) 0.015 COMMON PEAFOWL (PAVO CRISTATUS) 0.016 84.52 GREEN PEAFONL (PAYO MUTICUS) .017 73.25 0.057 GREAT ARGUS (ARGUSLANUS ARGUS) 0.045 BURMESE PEACOCK-PHEASANT (POLYPLECTRON BICALCARATUM) 0.050 'n BASE SUBSTITUTIONS PER SITE 15) GREEN PEAFOWL (PAVO MUTICUS) AA&&==CTTTTTTTAACCTAACCCCCCTACTGAGTGT&CCCCCCCTTTCCCCCCCAGGGGGGGTATAC 3) CHINESE BAMBOO PARTRIDGE (BAMBUSICOLA THORACICA) AATTTTCTTTTTTTAACCTAACCCCCCTATTGAGTGTACCCCCCCTTTCCCCCCCAGGGGÅGGTATAC 16) GREEN JUNGLEFOWL (GALLUS VARIUS) AATTTT=ÄTTTTTTAACCEAAC#CCCCTACTÄAGTGTACCCCCCCTTTCCCCCCCAGGGGGGGGTATAC 15) TATGCATAATCGTGCATACATTTATATACCACATAČATTATGGTČÄCÄGTAATAČATACTATATACGTACTAAACCCATTATATGTAĞACGGACATTACÄCTATČTTCCCCATTTÄTC 3) TATGTATAATCGTGCATACATTTATATACCACATATATTATGGTACCGGTAATATATACTATATACGTACTAAACCCATTATAČGTATACÅGACATTACTCTATATČCCÅCATTTCTC 241(307) CČACĨĂĊĊŽĂĊĂĂĠĊĂĊĊĊŢ<u>AĂĊŢĂŢĠĂĂŢĠŎŢĔ</u>ĂĊĂġĠĂĊĂŢĂĂĊĂĞŦŢĊĂĸŢĂŢŔĊŔĠ CŢĊŢĊĊĊĊ<u>ĂŢŢŢĠĠŢŢĂŢĠ</u>ĊŢĊĠŔĊĠŢĂŢĊAĠĂŢĠĠĂŢ ĨĂŢŦĊĂĊŢĂĂĊĂĠŢĊĂĊĊŢ<u>ĂĂĊŢĂŢĠĂŢĠĂŢĞ</u>ĂĊĠĠĠĂĊĂŢĂĂĂĊŢŢĂŢĂĊĂŢĠĊŢĊĊĊĊ<u>ĂŢŢŢĠĠŢŢĂŢĠ</u>ĊŢĊĠŔĊĠŢĂĊĊĂĠĬĠĠĂŢ ĊŢĂĊĊĊĊŢĂĂĊĂĞĞŢĊĂĊĊŢ<u>ĂĂĊŢĂŢĠĂŢĠĠŢŢ</u>ĂĊĂġĠĊĂŢĂĊĂĊĂŢŢĂĊĂŢĂŢĊŎĊŔŢĊĊĠĊĠŢĂĊĊĠĂŢĠĠĂŢ 15) CCCACGTTC 192 3) CCCATGTAC 16) CCCATGTAC 3) TTATTGATCGTACACCTCACGAGAGA&CCAGCCAACCCCTGCCTGTAATGTACTTCATGACTAG&CTCAGGCCCATTCTTTCCCCCCTACACCCCTGC 16) TTATTGATCGTECACCTCACGAGAGATCAGCAACCCCTGCCEGTAATGTACTTCATGACEAGFCTCAGGCCCATTCTTTCCCCCTACACCCFTCGC IDENTITY DIFFERENCES TRANSITIONS TRANSVERSIONS GAPS BAMBUSICOLA THORACICA 85,71% (336/392) 13 20 23 COTURNIX COTURNIX J PAVO MUTICUS 86.82% (336/387) 5 21 25

 GALLUS VARIUS
 BAMBUSICOLA IHORACICA
 88.37% (342/387)
 2
 20
 23

 BAMBUSICOLA IHORACICA
 88.37% (342/387)
 2
 20
 23

 BAMBUSICOLA IHORACICA
 89.15% (345/387)
 3
 20
 19

FIG. 2. (*Top*) The last row of the phasianine mitochondrial sequence alignments is followed by the dendrogram of 12 phasianine species. (*Middle*) Sequence of the Chinese bamboo partridge (*B. thoracica*) is aligned with the sequence of the green peacock (*P. muticus*) and that of a green junglefowl (*G. varius*). (*Bottom*) Identity and differences between four pertinent pairs of species. Each identity is shown as a percentage, as well as a number of identical sites per the total of 392 in parentheses. Differences are shown as numbers of gaps, transitions, and transversions. GenBank accession numbers are as follows: D66888, blue-breasted quail; D66889, Chinese bamboo partridge; D66890, chukar partridge; D66891, European grey partridge; D66897, silver lophura; D66893, Sri Lanka junglefowl; D66894, ring-necked pheasant; D66895, golden pheasant; D66896, Mrs. Hume's pheasant; D66897, silver lophura; D66898, great argus; D66899, Burmese peacock-pheasant; D66900, common peafowl; D64164, green peafowl; D64163, green junglefowl.

upon numbers of copies of the 60-base-long unit individual birds possessed.

The Antiquity of Generalized Perdicine Lineages (Quails and Partridges). Differences at 392 positions of the mitochondrial noncoding control region of five perdicine species are shown in Fig. 1 Top, and the dendrogram drawn based upon these differences is shown immediately below. It would be noted that the European grey partridge (Perdix perdix p.) was separated from the rest by the greatest distance. The second in remoteness was the chukar partridge (Alectoris graeca chukar). Although the remaining three appeared to be cohorts of one cohesive group, the closest relationship was seen within a pair consisting of representatives of two separate tribes, C. coturnix j. (6) representing the tribe Corturnicini and B. thoracica of the tribe perdicini (2). Regardless of whether the common quail and the blue-breasted quail belong to the same genus Coturnix or to two separate genera, Coturnix and Excalifactoria, the two belonged to the same tribe. Yet, the distance separating the two appeared slightly greater than that between the common quail and the Chinese bamboo partridge in the perdicine dendrogram of Fig. 1. At any rate, even the closest pair of perdicine species shared identical bases at only 336 of the 392 positions (mere 85.71% identity), as shown in Fig. 2 Bottom. There would be no better testimony than the above as to the antiquity of each generalized perdicine lineage.

Three Distinct Branches of Phasianine Birds. The base sequences of the nine phasianine species are aligned in four rows from Fig. 1 Bottom to Fig. 2 Top. Adding the base sequences of red and green junglefowls (G. gallus and G. varius) previously published (1), as well as the sequence of the green peafowl (P. muticus) shown separately in Fig. 2 Bottom, to the present comparison, the dendrogram of 12 phasianine species was drawn and shown in Fig. 2. It would immediately be seen that the first major division separated the branch made of peafowls of the genus Pavo, the great argus, and the peacock-pheasant from the rest of phasianine birds. These remainders were further subdivided into two branches. One was made exclusively of members of the genus Gallus, while the other was a pheasant branch. In addition to those belonging to genera Phasianus, Chrysolophus, and Syrmaticus, gallopheasants of the genus Lophura were included in this branch. Our phasianine dendrogram shown in Fig. 2 agrees essentially with that previously produced on the basis of classical taxonomical studies (2). Unfortunately, those that are thought to be members of the fourth independent branch were not included in the present study; they were tragopans, monals (Lophophorus), and the koklass (Pucrasia) (2).

**Perdicine Versus Phasianine Lineages.** We made a number of attempts to construct a combined dendrogram of perdicine and phasianine species studied. In the process, it was found that when two rather distantly related groups are combined, relative positions of individual species in a dendrogram tend to shift by an addition to or a subtraction from that diagram of a few species. Because our survey is by no means comprehensive, lacking representation from a few key groups as already noted, we concluded that the construction of a combined diagram at this time would be inadvisable.

One Tangible but Paradoxical Genetic Link Between the Chinese Bamboo Partridge and Members of the Genera Gallus and Pavo. In spite of an extreme meagerness of perdicine species in the present survey, one tangible link emerged between perdicine and phasianine lineages, but this link was a paradoxical one. In Fig. 2 Bottom, the mitochondrial DNA base sequence of B. thoracia is aligned with that of the green peafowl (P. muticus) on one hand and that of the green junglefowl (G. varius) on the other. Pertinent information extracted from these alignments is shown immediately below them. The Chinese bamboo partridge is genetically far closer to phasianine *Gallus* (89.15% identity) and *Pavo* (88.37% identity) than to its closest perdicine ally, *Coturnix coturnix j.* (85.71% identity).

A paradox is found in the fact that Gallus and Pavo belonged to two very divergent phasiane lineages, as evident in the dendrogram of Fig. 2, as well as in only 85.02% sequence identity between G. varius and P. muticus (Fig. 2 Bottom). While nearly equal affinities toward B. thoracica were shown by all four species of Gallus, as well as by both species of Pavo, no hint of a close link was seen between Bambusicola and other members of the peafowl branch—i.e., Argusianus argus and Polyplectron bicalcaratum.

## DISCUSSION

Poul A. Johnsgard, in his authoritative treatise on the subject "The Pheasants of the World," follows the customary subdivision of the subfamily Phasianinae to Perdicini and Phasinanini (2). Yet, he is of the opinion that four branches of phasianine birds sprung independently from generalized perdicine ancestors. This view is succinctly illustrated in his dendrogram shown as figure 1 of ref. 2. Indeed, the present study established one direct link between a particular perdicine lineage represented by B. thoracica and two phasianine genera Gallus and Pavo. The only problem was that Gallus and Pavo belonged to two very divergent phasianine branches. It is quite conceivable that Bambusicola with only two extant species (B. thoracica and B. fytchii) belong to one-stem perdicine lineage, which later splits into two phasianine-yielding sublineagesone being ancestral to Gallus, while the other gives rise to Pavo and its allies. If so, one might expect to locate a species or species belonging to one or the other of the above noted two phasianine-yielding sublineages among close relatives of Bambusicola. The most attractive in this respect are three species of Galloperdix. If the implication from its generic name holds true, they might show even closer affinity toward Gallus than Bambusicola, while manifesting little affinity toward Pavo. Unfortunately, all attempts to secure samples of Galloperdix inhabiting India and Sri Lanka failed.

At any rate, there is an independent line of evidence that links *Bambusicola* with *Gallus*. In 1949, Yamashina classified the chromosome complements of members of the subfamily *Phasianinae* to four major types. The karyotype of *Bambusicola, Coturnix*, and *Gallus* belonged to the same type III. However, *Pavo* belonged to the type IV (7).

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