

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

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**ABSTRACT** Further comparison of mitochondrial control-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. Grouse 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 prop-

agated 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 sinensis*, 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 ring-necked (*Phasianus colchicus*), the golden (*Chrysolophus pictus*), and Mrs. Hume's (*Syrmaticus 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 peacock-pheasant (*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 neighbor-joining 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).

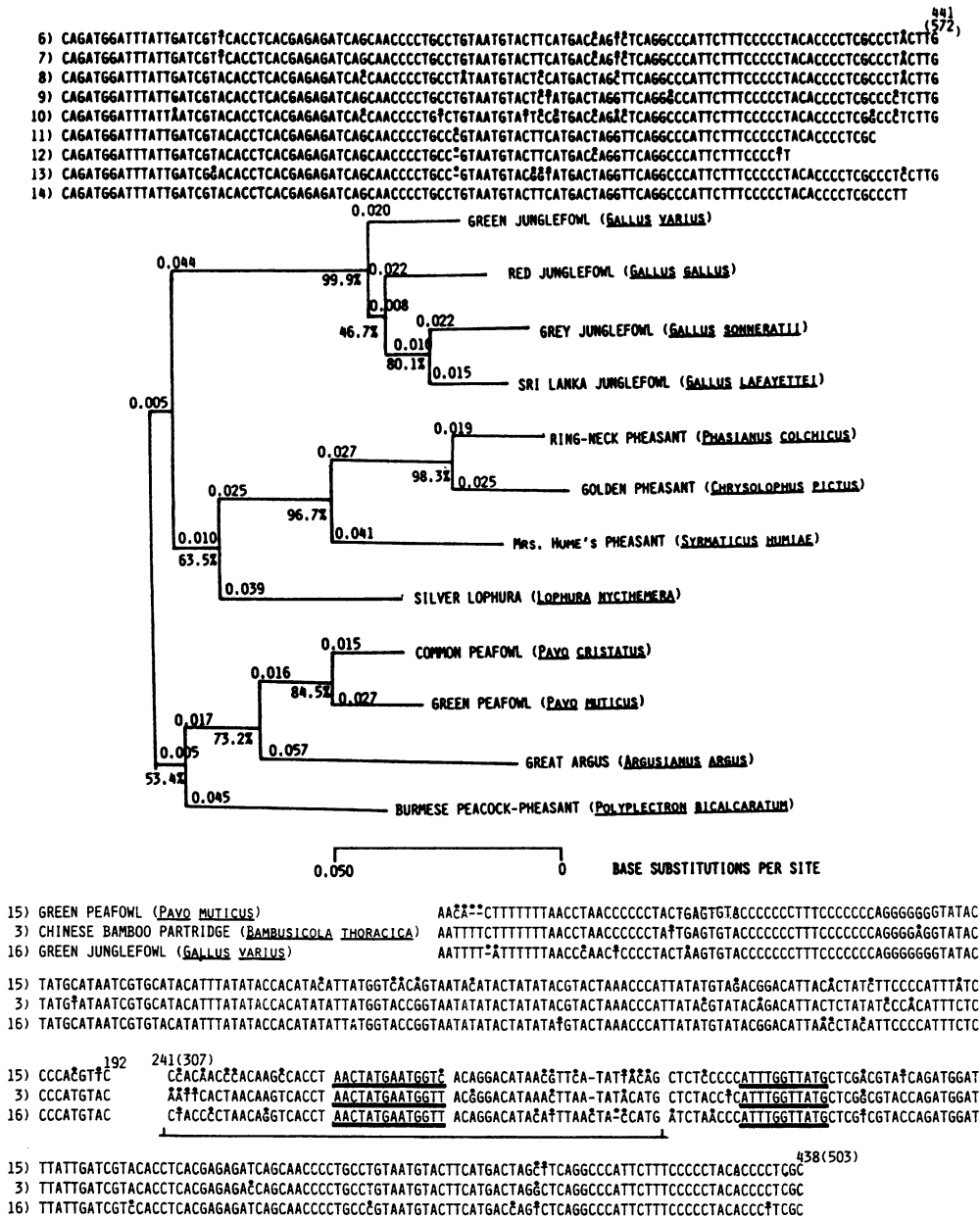
### OBSERVATIONS

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>The sequences reported in this paper have been deposited in the GenBank data base (accession nos. D64163, D64164, D66888–D66900).





	IDENTITY	DIFFERENCES		
		GAPS	TRANSITIONS	TRANSVERSIONS
BAMBUSICOLA THORACICA	85.71% (336/392)	13	20	23
COTURNIX COTURNIX L.				
PAVO MUTICUS	86.82% (336/387)	5	21	25
GALLUS VARIUS				
BAMBUSICOLA THORACICA	88.37% (342/387)	2	20	23
PAVO MUTICUS				
BAMBUSICOLA THORACICA	89.15% (345/387)	3	20	19
GALLUS VARIUS				

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; D66892, grey junglefowl; 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 *Coturnicini* 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. thoracica* 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 phasianine 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 *Phasianini* (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 sublineages—one 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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