Comparative anatomy of the hepatobiliary systems in quail and pigeon, with a perspective for the gallbladder-loss.

Hiroki HIGASHIYAMA¹, Yoshiakira KANAI²

¹ Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

² Department of Veterinary Anatomy, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

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* correspondence to:
Hiroki Higashiyama
Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan.
Email: higashiyama.hiroki@gmail.com
Tel: +81-3-5841-3496
Although the gallbladder is one of the characteristic component of the vertebrate body, it has been independently lost in several lineages of mammals and birds. Gallbladder loss is a widely reported phenomenon; however, there have been few descriptive comparisons of entire hepatobiliary structures between birds with and without a gallbladder. Here, we discuss the evolution of avian hepatobiliary morphology by describing the gross anatomy of the hepatobiliary system in the quail and pigeon. Quails have two major extrahepatic bile ducts: the right cystic-enteric duct, which has a gallbladder, and the left hepatic-enteric duct, which does not. Together with two pancreatic ducts, they share one opening to the ascending part of duodenum. Pigeons lack a gallbladder, but also have two extrahepatic ducts similar to those of quails. However, the hepatic-enteric duct opens solely to the descending part of the duodenum close to the stomach. The pancreatic duct opens to the very posterior part of the duodenum independent from the biliary tracts, giving rise to three separate openings in the duodenum. The hepatobiliary anatomy of the pigeon represents a highly derived condition not only because of gallbladder loss. Avian gallbladder loss may be related to remodeling of the entire hepatobiliary system, and may have occurred via a different mechanism from that of mammals, which can be explained simply by the disappearance of the gallbladder primordium.

KEY WORDS: biliary tract, bird, gallbladder, pigeon, quail
INTRODUCTION

The liver and related structures are essential visceral components of vertebrates [19, 34]. The liver, pancreas and bile ducts are collectively called the hepatobiliary system (or hepatopancreatic system), and vary anatomically among vertebrates. However, there have been few comparative morphological studies. One well-known but mysterious phenomenon is gallbladder loss. The gallbladder is a bladder-shaped organ that stores bile fluids in the biliary tract [16]. This organ has a unique smooth muscle layer that produces the contractile movement necessary for bile flow and, phylogenetically, is considered an ancestral character of vertebrates [31]. However, the gallbladder has been independently lost by many therian mammals and birds for unknown reasons [9, 14, 30]. In the case of mammals, a previous study reported that gallbladder loss occurred due to the loss of the gallbladder–cystic duct domain, which corresponds to the biliary bud in the pharyngula embryo [14]. In birds, however, even anatomical differences between those with and without a gallbladder remain uncertain.

Morphological evolutionary studies of the hepatobiliary system are less well-established than those of other body systems, such as the musculoskeletal system. In mammals, the most detailed studies have been done on domestic animals, such as rodents, cats, dogs, shrews, and horses, as well as humans [3, 12-15, 25-28, 43]. Descriptions of non-mammalian groups are usually oversimplified, and related structures such as vessels are omitted. Thus, it remains difficult to conduct comparative anatomical studies of different vertebrate classes. Avian hepatobiliary anatomy has been documented in some reviews and textbooks [38, 41]. The most
well-described species are domestic animals, such as chickens, quails, and geese [6, 8, 42]. However, the descriptions are focused on individual species, and comparative studies are rare. Therefore, questions remain regarding avian hepatobiliary anatomy. Although gallbladder loss is known to have occurred in many lineages (e.g., ostriches, pigeons and other Columbiformes, falcons, parrots, and some species in Passeridae; [9]), the morphological changes in the hepatobiliary system that accompany this phenomenon have not been well documented in comparative anatomy studies.

In the present study, we performed whole-mount anatomical comparisons between the hepatobiliary systems of the quail (with a gallbladder) and pigeon (without a gallbladder). Although only two species were compared, the comparison was as detailed as possible, and included the vascular system. We then compared our findings with previous studies in a discussion of hepatobiliary evolution in birds.
MATERIALS AND METHODS

Animal collections

We observed four quails (*Coturnix japonica*) and three pigeons (*Columba livia*). These specimens were already fixed with formalin, and had been held at the Laboratory of Veterinary Anatomy, University of Tokyo. Colored latex was already injected into the blood vessels before fixation. We assigned these specimens IDs, as follows. Quail: UTVA139-B001 (width of left liver lobe [WL] 365 mm, female), UTVA140-B002 (WL 330 mm, male), UTVA141-B003 (WL 302 mm, male), and UTVA142-B004 (WL 409 mm, female). Pigeon: UTVA143-B005 (WL 512 mm, female), UTVA144-B006 (WL 498 mm, male), and UTVA145-B007 (WL 534 mm, male). The ages of individuals, and where they were obtained, were not recorded. To prepare the specimens for observation, we immersed them in tap water for several weeks to remove the formalin. After that, the samples were made transparent using CUBIC solution following previous studies [13]. This reagent can easily make opaque tissues (including of the liver and pancreas) transparent, and is easy to handle because it is not an organic solvent. Because the specimens were old, the livers were not highly transparent, but transparency was nevertheless sufficient to observe the extrahepatic biliary tracts.

Terminology

The nomenclature used here was based on descriptions of domestic fowl provided in previous studies [32, 33]. The comparative studies were based on a previous paper [13] and the references therein. In particular, the literature indicated that there are two main avian bile ducts: the hepatic-enteric duct (ductus hepatoeentricus), located anteriorly and not having a gallbladder, and the cystic-enteric duct (ductus cysticoentericus). Since this “double biliary duct” is not seen in other animals, we paid special attention to it in this study.
RESULTS

Quail

In quails, the liver is composed of left and right lobes. The gallbladder and extrahepatic biliary tract were found on the caudal side of the liver (Fig. 1A, B). In contrast to the mammalian biliary tract, which has only one duct, the quail tract has two major ducts: the cystic-enteric duct (*ductus cysticoentericus*) and hepatic-enteric duct (*ductus hepatoentericus*) [32, 33]. The ducts start from the middle part of the liver, between the left and right lobes, and run in parallel straight toward the duodenum. No small branches are present. Then, both biliary ducts open to the ascending part of the duodenum (*duodenum, pars ascendens*) at the same position (Fig. 1D, E). Those two ducts do not create anastomoses in the extrahepatic area. The gallbladder connects only to the cystic-enteric duct. The dorsal and ventral pancreatic ducts also arise from the duodenal opening of these two biliary ducts (Fig. 1D, E). The dorsal pancreatic duct continues to the pancreatic lobe, which attaches to the descending part of the duodenum, while the ventral duct attaches to the ascending part.
All major arteries of the hepatobiliary system arise from the trunk of the celiac artery (Fig. 1C, E). Two major arteries branch from the celiac trunk: the common hepatic artery (*Arteria hepatica communis*) and the left gastric artery (*Arteria gastrica sinistra*). The former continues directly to the right hepatic artery (*Arteria hepatica dextra*) and then supplies the biliary tract. The gastroduodenal artery (*Arteria gastroduodenalis*) also branches from the common hepatic artery, to supply the duodenum and pancreas.

The portal vein supplies the hepatobiliary system. Corresponding to the long duodenum, the pancreaticoduodenal vein (*Vena pancreaticoduodenalis*) is large and supplies many small branches in the duodenum. The distal part of the hepatic-enteric duct runs in parallel with the portal vein, but other parts do not (Fig. 1E).

**Pigeon**

Similar to the quail liver, the pigeon liver has obvious left and right lobes (Fig. 2A). However, pigeons have no gallbladder. As seen in the quail, two extrahepatic biliary ducts were observed caudally in the liver, which we similarly termed the cystic-enteric and hepatic-enteric ducts. Their ascent from the liver is similar to that in quails, i.e., starts in the middle part between the left and right lobes. However, the positions of the duodenal openings are very different. The cystic-enteric duct opens to the ascending part of the duodenum (*duodenum, pars ascendens*), which is at a similar level to the duodenal openings in the quail. The hepatic-enteric duct opens to the anterior-most level of the descending part of the duodenum (*duodenum, pars descendens*), which is immediately caudal to the muscular stomach (Fig. 2D, E). Hence, unlike in the quail, the two
extrahepatic bile ductal trunks do not open to the duodenum at the same positions, but rather are very far from each other. Furthermore, the pancreatic ductal opening also differs from that of the quail, making an anastomosis with the duodenum at the posterior-most level in the ascending part of the duodenum, which is much more posterior to the cystic-enteric ductal opening (Fig. 2E).

Despite the differences in biliary tract anatomy, the arteries branch from the trunk of the celiac artery. In contrast to quails, the right hepatic artery (*Arteria hepatica dextra*) is large and enters the liver directly. After entering the liver tissue, it immediately branches into small arteries that supply the extrahepatic biliary tract (Fig. 2E). The gastroduodenal artery branches from the celiac trunk, which passes caudally to the spleen.

All venous supply was from the portal vein. Similar to quails, pigeons also have a long pancreaticoduodenal vein (*Vena pancreaticoduodenalis*). Additionally, the distal part of the hepatic-enteric duct runs in parallel with the portal vein just before entering the liver (Fig. 2E).
DISCUSSION

In the present study, we observed the anatomy of the hepatobiliary systems of the quail and pigeon. The systems were the same in terms of the topographical relationships of the blood vessels, duodenum, liver, and pancreas; both also exhibited a “double biliary duct”. However, the position of the duodenal opening differed. Previous studies suggest that the quail-type biliary duct represents the ancestral avian condition (see below); thus, the condition in the pigeon is derived. Nevertheless, it is difficult to determine whether biliary differences are related to gallbladder loss. Further research of closely related species of pigeons is thus warranted. Regardless, the avian double trunk system of the biliary anatomy is highly derived among vertebrates, and it should be noted that the evolutionary processes leading to gallbladder loss may differ from those of mammals.

Comparison of the quail and pigeon

We found many anatomical differences between the hepatobiliary systems of the quail and pigeon (Fig. 3). In the quail, the two bile ducts and the pancreatic ducts shared the same duodenal openings, whereas in the pigeon each duct opened to the duodenum at a different level. Thus, in addition to gallbladder status (i.e., present vs. absent), the entire morphology of the biliary system differed. However, it is not clear whether gallbladder loss is related to differences in the biliary tract. Biliary tract morphology is somehow a most plastically changeable part among the vertebrate body. For example, many ductal variations exist among rodents with a gallbladder. In some cases, branching of the biliary tract is seen (e.g., chinchillas [29]), while in others the duodenal opening of the pancreatic
duct is entirely separated from that of the biliary duct, although these ducts share the same
opening in many rodents (e.g., prairie dogs and guinea pigs [10]). This variation in
connectivity is considered to be unrelated to dietary and life history factors. Despite these
complexities, comparison of our results with previous studies should help clarify the
evolutionary trends of the avian biliary system and it reveal whether the quail or the
pigeon system is more ancestral.

The topography of the hepatobiliary system of the quail, as revealed in the
present study, is highly conserved with that of the closely related chicken [32, 33].
Despite the lack of information on blood vessels, the branching pattern of the biliary tract
observed in the present quails is also conserved among several species of duck [23].
Yamagishi [41] conducted a comparative study of the chicken (Gallus gallus; “Gallus
domesticus”), pigeon (Columba livia; “Columba intermedia”), tree sparrow (Passer
montanus), Japanese bush warbler (Horornis diphone; “Horeihus cantous”), brambling
(Fringilla montifringilla), Japanese quail (Coturnix japonica), duck (Anas
platyrhynchos; “Anas domestica”), and little egret (Egretta garzetta; “Herodias
garjetta”). These species exhibit two biliary ducts. Although the positions of bile trunks
and pancreatic ductal openings vary slightly in the duodenum, almost all branches arise
from the ascending part of the duodenum. This means that the system observed in the
pigeon is highly derived, not only in terms of gallbladder loss but also in terms of the
branching pattern of the biliary tract.
The ostrich also lacks a gallbladder, and its anatomy appears to represent a more
extreme form of the pigeon-type hepatobiliary morphology. The ostrich biliary tract has
only a hepatic-enteric duct, which opens to the anterior-most level of the descending part
of the duodenum, as seen in the pigeon [1, 24]. The pancreatic duct opens to the
ascending part of the duodenum separate from the biliary duct [1]. Hence, although the
blood-vessel pattern remains unclear, the hepatobiliary system of the ostrich is largely the
same as that of the pigeon, with the exception of the cystic-enteric duct.

The above comparisons suggest that avian gallbladder loss may be related to
changes in the connectivity of the biliary tract itself. However, given the plasticity of the
biliary tract in rodents, this hypothesis requires additional evidence. To further evaluate
gallbladder loss in birds, morphological data are needed from columbiform taxa that have
not lost their gallbladder, such as *Ptilinopus, Ducula,* and *Gymnophaps* [7, 11].

**Peculiarities of the avian hepatobiliary morphology**

In birds, as in mammals, the arteries of the hepatobiliary system are supplied
by the celiac trunk, and the venous system by the portal vein. Although the peripheral
vessels in this region tend to show remarkable variation even within the same species [13,
21, 25-27], at least in the major vascular trunks, the avian vasculature is generally the
same as that of mammals (Fig. 3). The most characteristic feature of the hepatobiliary
system of birds is the presence of two biliary ducts. Phylogenetically, this double biliary
trunk is a derived condition, as the other amniotes (e.g., turtles, lizards, and mammals)
exhibit a single ductal trunk. The crocodilians, which are in the same Archosaurus lineage
as birds, “nearly” have a double biliary duct (e.g., the American alligator; *Alligator
mississippiensis*) [40]. The most common pattern of *A. mississippiensis* differs
significantly from birds by having the multiple anastomoses between the two bile trunks.
However, there is fluctuation in morphology within the same species, and a few percent
of individuals represent a separated double-trunk condition, a similar pattern to birds [40].

Hence, the double biliary duct appears to have been sequentially established within the archosaurian lineage.

Along with the double biliary duct, the developmental process also appears to be highly derived in birds. Despite wide variation in the early developmental stages [4], the morphology of the hepatobiliary primordia is primarily conserved in the pharyngula period. The hepatobiliary system first arises from the ventral side of the posterior foregut as a hepatic diverticulum [37]. Then, in non-avian vertebrates, one biliary and several hepatic buds arise from the diverticulum. Simultaneously, a ventral pancreatic bud develops in the region caudal to the biliary bud, and a dorsal pancreatic bud arises from the dorsal side of the foregut (Fig. 3) [14, 37 and references therein]. There is usually one ventral pancreatic bud, but it is sometimes divided into left and right parts (e.g., in echidnas, geckos, and birds) [5, 20]. The number of hepatic buds varies; however, they are typically clustered within a single duct throughout development (i.e., the common hepatic duct) and continue directly to the biliary duct [35, 39]. In any case, gallbladder loss in mammals is thought to be due to the absence of a biliary bud during the entire developmental process [14].

Meanwhile, in birds, there are two hepatobiliary buds (variously referred to as the Lebergang or Ductus hepaticus). These are separated craniocaudally; the cranial one develops into the hepato-enteric duct, and the caudal one (including a biliary bud) into the cystic-enteric duct (Fig. 3). This has been observed in various avian species, including chicken [2], quail (Coturnix japonica) [22], and black-tailed gull (Larus crassirostris) [18]. It is unclear how these two hepatobiliary buds compare to the ancestral biliary and
hepatic buds (Fig. 3). Even in pigeons, these primordia are present during development and show the same topography [36]. Furthermore, during the embryonic stage in pigeon, an apparent biliary bud (gallbladder) arises from the posterior hepatic duct (cystic-enteric duct in the present study); the gallbladder “degenerates” later during development [36].

Thus, avian gallbladder loss reflects secondary degeneration of the biliary bud during development, similar to eye loss in cavefish [17] but differing from that of rodents (e.g., rats), whose biliary bud never arises during development [14]. This also differs from the lamprey, which loses its gallbladder with maturation [36, 44]. Therefore, gallbladder loss in mammals and birds may not occur via the same developmental changes. It is unclear whether there is a common molecular mechanism underlying the maintenance or formation of the gallbladder primordium; however, even if the mechanisms were similar, timing with respect to their effects may differ between these two animal groups.

In summary, we compared the anatomy of the hepatobiliary systems of the quail and pigeon to elucidate the various problems related to avian gallbladder loss. Understanding the development of the caudal hepatobiliary buds (future cystic-enteric ducts) is in turn critical to understanding the phenomenon of gallbladder loss itself. However, it remains difficult to determine which factors caused gallbladder loss in birds and mammals alone among the vertebrates. There are numerous difficulties associated with comparing the entire hepatobiliary systems of birds and other lineages, mostly because of the double biliary duct (which may be unique to avian evolution). A focus on comparative anatomy and development in future studies could resolve many questions regarding the evolution of the hepatobiliary system.
CONFLICT OF INTERESTS

The authors have no conflict of interest to declare.

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REFERENCES


Figure 1.
The gross anatomy of the extrahepatic biliary system in the quail. Colored latex (blue) was injected into the portal vein before fixation. (A) Ventral view of the viscera. (B–D) Enlarged views of the viscera highlighted in (E). Abbreviations: a.gmd, *Arteria gastrica muscularis dextra* (right muscular gastric artery); a.gs, *Arteria gastrica sinistra* (left gastric artery); a.hd, *Arteria hepatica dextra* (right hepatic artery); v.gmd, *Vena gastrica muscularis dextra* (right muscular gastric vein); v.mcr, *Vena mesenterica cranialis* (cranial mesenteric vein); v.pad, *Vena pancreaticoduodenalis* (pancreaticoduodenal vein); v.pd, *Vena portae dextra* (right portal vein).
Figure 2

The gross anatomy of the extrahepatic biliary system in the pigeon. Colored latex (blue) was injected into the portal vein before fixation. (A) Ventral view of the viscera. (B–D) Enlarged views of the viscera highlighted in (E). Abbreviations:
a.gmd, *Arteria gastrica muscularis dextra* (right muscular gastric artery); a.gs, *Arteria gastrica sinistra* (left gastric artery); a.hd, *Arteria hepatica dextra* (right hepatic artery); v.gmd, *Vena gastrica muscularis dextra* (right muscular gastric vein); v.mcr, *Vena mesenterica cranialis* (cranial mesenteric vein); v.pad, *Vena pancreaticoduodenalis* (pancreaticoduodenal vein); v.pd, *Vena portae dextra* (right portal vein).
Figure 3
Schematic comparison of the quail, pigeon, and mammalian hepatobiliary systems. The mammalian system was drawn based on a previous study [13]. It is difficult to compare the double biliary duct of birds with the single biliary duct of mammals. Considering the embryonic primordia, we hypothesize three evolutionary processes. The first is that the entire caudal hepatobiliary bud corresponds to both the ancestral hepatic and biliary buds; thus, the cranial hepatobiliary bud is a novelty in the archosaurian lineage. The second hypothesis is that the cranial and caudal hepatobiliary buds correspond to the ancestral hepatic and biliary buds, respectively; thus, the biliary bud newly acquired the ductal connection to the liver tissue. The third hypothesis is that the ancestral hepatic bud duplicates and separates craniocaudally over a long distance. Regardless, avian gallbladder loss can be explained mainly as a change in the caudal hepatobiliary bud, at least for pigeons. In fact, avian gallbladder loss reflects secondary degeneration of the biliary bud, unlike in rats, which do not have a biliary bud during development.