CHAPTER 8

NON-NEOTENOUS ORIGIN OF THE PALAEOGNATHOUS PTERYGOID-PALATE COMPLEX

Summary

Recent studies on the phylogeny of modern birds have indicated that the Palaeognathae may not be the most basal group in modern birds, but are derived within the group. This resulted in the revival of the theory that the Palaeognathae have evolved through neoteny from a flying ancestor. Neoteny was also suggested after finding palaeognath-like characters in neognathous birds after experimentally induced neoteny (neonatal thyroidectomy). In this study we test whether the most important palaeognathous character, the palaeognathous Pterygoid-Palate Complex (PPC), has evolved through neoteny. We used a numerical method to compare the morphology of the PPC of adult Palaeognathae with the PPC of several stages of development of neognathous birds. The results show that the morphology of the palaeognathous PPC is very different from ontogenetic stages of the chicken, indicating a non-neotenous origin of the palaeognathous PPC.

Introduction

The taxon Palaeognathae consists of the Ostrich, the Rheas, the Emu, the Cassowaries and the Kiwis, all non-flying birds from the southern hemisphere (Sibley & Monroe, 1990; Cracraft, 1974). The poor-flying Tinamous are often also included in the taxon (Sibley & Monroe, 1990). The evolution of the Palaeognathae has puzzled biologists for years, especially since the number of species is relatively low, and their distribution restricted to the southern hemisphere. It has often been questioned whether the taxon is monophyletic, and if so what the evolutionary background of the group is. The monophyly of the group is supported both by molecular and morphological data (Cracraft, 1974; Bledsoe, 1988; Sibley & Ahlquist, 1990; Mindell *et al.*, 1997) and is now generally accepted. The phylogenetic position of the taxon is, however, less clear. Two main hypotheses are proposed to explain the evolution of the palaeognathous birds. The first states that the Palaeognathae are the earliest offshoot in the evolution of modern birds (Neornithes; Cracraft, 1974; Feduccia, 1995). After this bifurcation the morphology of the Palaeognathae has changed only slightly and several apparently primitive characters are still present in their morphology (Olson, 1985; McDowell, 1948). The alternative hypothesis states that the Palaeognathae are a relatively young taxon, which has only recently evolved (de Beer, 1956; Gingerich, 1976, Jollie, 1976; Härlid & Arnason, 1999). The apparent primitive features in the morphology of the Palaeognathae are thought to be the result of neoteny (de Beer, 1956; Jollie, 1976). Although the neoteny hypothesis has had little support, recent research points into the same direction. Two independent molecular phylogenetic analyses of birds showed that the position of the Palaeognathae is not basal within modern birds, but more derived (Mindell *et al.,* 1997; Mindell *et al.*, 1999; Härlid & Arnason, 1999). In addition to this molecular phylogenetic argument a physiological experiment showed that palaeognathous characters appear in adult neognathous birds after neonatal thyroidectomy (Dawson *et al.*, 1994). This thyroidectomy results in growing disorders considered similar to neoteny. After thyroidectomy the palate of a neognathous bird shows a reduced development of the lateral process and a reduced fusion of elements. Dawson *et al.* consider this similar to the condition found in adult Palaeognathae and see their results as an indication for a neotenous origin of the Palaeognathae.

Based on these findings a morphological analysis will be made to test whether the characters that are specific for the Palaeognathae can be found in a developmental series of a neognathous bird. For this morphometric analysis one of the most distinctive characters of the Palaeognathae was chosen: the palaeognathous Palate-Pterygoid Complex (Gussekloo & Zweers, 1999). This complex is situated in the dorsal wall of the buccal cavity and consists of a series of bony elements, including pterygoids, palates and vomer. The morphology of this palaeognathous system was described in detail by McDowell (1948). He concluded that the differences in the morphology of the PPC between the different members of the Palaeognathae were too large to call it an unique character. This was later disputed by Bock (1963) who stated that differences within the Palaeognathae are less than the differences between the palaeognathous condition and the neognathous condition. Bock therefore concluded that the palaeognathous PPC can be defined and is unique for to the Palaeognathae. Recently, this was confirmed using a numerical method (Gussekloo & Zweers, 1999). Using several quantitative measurements of the PPC in a discriminant analysis a clear difference between palaeognathous and neognathous birds was found. This indicated that the palaeognathous PPC is indeed a unique character.

To test the hypothesis that the palaeognathous PPC has evolved through neoteny the morphology of adult palaeognathous birds is compared to the early stages of development of a neognathous bird. The morphology was described using a multivariate method to simultaneously analyse as much information as possible. We hypothesise that, if the palaeognathous PPC is the result of neoteny, the shape of the PPC in chicken embryos should be more similar to the shape of that of Palaeognathae than to that of Neognathae. The chicken (*Gallus gallus*) was chosen as representative for the neognathous birds because much is known about the development of this species (Hamburger & Hamilton, 1951; Bellairs & Osmond, 1998) and it belongs to one of the most basal groups within the Neognathae (Cracraft, 1974; Sibley &

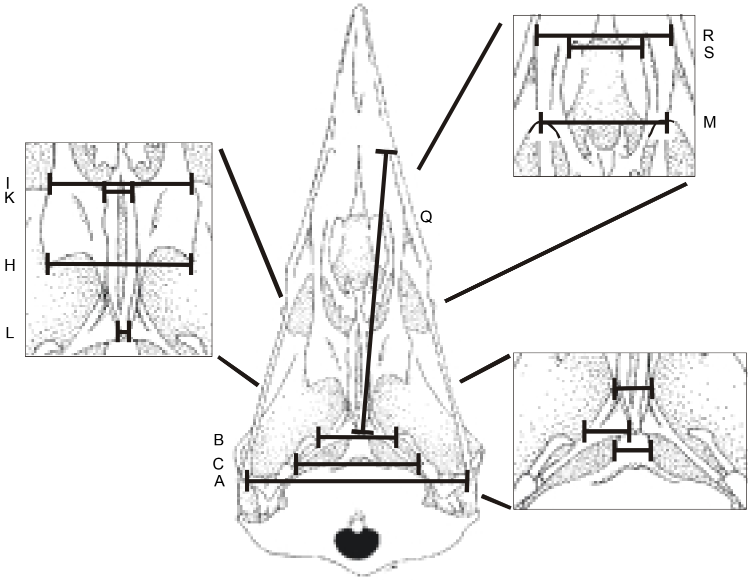

Figure 8.1. Skull of the crow (Corvus corone) in ventral view. Inserts are enlargements of areas indicated by the lines. Letters refer to characters in table 8.1. The characters of the vomer cannot be represented since the vomer is reduced in this species. Other characters omitted from this figure for clarity are: O, P, and L. Characters O and P are measured in the sagital plane. Character L is measured at the most caudal point of the pterygoid-palatine articulation.

Table 8.1. Character used for distance analysis including contribution of each parameter to the first principle component (component loadings).

-	**Character**	**Value PC1 (variation: 40%)**
A	Skull width at the quadrate-jugal articulation [standard]	
B	Distance between most distal points of Processi orbitalis quadrati	0.472
C	Width at pterygoids at quadrate-pterygoid articulation	0.754
D	Width of pterygoids at pterygoid-palate articulation	0.753
E	Maximal width of the right pterygoid in the transversal plane	-0.248
F	Width of the vomer [caudal]	0.891
G	Width of the vomer [rostral]	0.893
H	Width of the caudal part of the palatal wings (pars lateralis)	0.841
I	Maximal width of the palate medial ending of pars lateralis	0.743
K	Width between palates at position 'I'	0.853
L	Width of palate at pterygoid-palate articulation	0.917
M	Internal width at jugal-premaxillae articulation	-0.073
N	Width of the *R. parasphenoidale* incl. *P. basipterygoidei* if present	0.324
O	Distance Foramen magnum to measurement 'N'	-0.126
P	Distance Foramen magnum to medial fusion of bony elements	0.113
Q	Maximal length palate	0.343
R	Width at palate-premaxillae articulation	0.308
S	Internal width at palate-premaxillae articulation	0.155

Ahlquist, 1990). In addition to the embryonic specimens of the chicken, several adult neognathous species from 7 different orders were added to the analysis, to get an overview of diversity in morphology within the Neognathae.

Materials and Methods

The PPC characters measured were described earlier in Gussekloo & Zweers (1999). The exact characters are also given in table 8.1 and figure 8.1. Characters were measured in a group of adult Palaeognathae and for comparison in a group of neognathous birds (Table 8.2). The characters of the adult Palaeognathae and Neognathae were measured on osteological specimens using a digital calliper rule (Sylvac, accuracy 0.01mm). Measurements were taken twice on each specimen and averages were used for further analysis.

The developmental series of the chicken consisted of cranial specimens of embryos in developmental stages from 10 days after incubation (stage 36, Hamburger & Hamilton, 1951) up to hatching (n=2 per day of development), 20 days after incubation (stage 46, Hamburger & Hamilton, 1951). Chicken eggs (White-Leghorn variety) were obtained from a commercial breeder and incubated in an automated incubator (37 ^{o}C, >90% air humidity.). After day nine, each day two eggs were taken from the incubator and the embryos removed from the egg. The embryos were then preserved in 4% formaldehyde.

Table 8.2. Species used in distance analysis. Names according to the classification of Sibley and Monroe (1990,1993).

No	Order	Family	Species	Common name
1	Struthioniformes	Struthionidae	*Struthio camelus*	Ostrich
2		Rheidae	*Rhea americana*	Greater Rhea
3		Casuariidae	*Casuarius casuarius*	Southern Cassowary
4		Casuariidae	*Dromaius novaehollandiae*	Emu
5		Apterygidae	*Apteryx owenii*	Little spotted Kiwi
6	Tinamiformes	Tinamidae	*Rhynchotus rufescens*	Red-winged Tinamou
7	Galliformes	Phasianidae	*Gallus gallus domesticus*	Chicken
8		Phasianidae	*Phasianus colchicus*	Common Pheasant
9	Anseriformes	Anhimidae	*Anhima cornuta*	Horned Screamer
10		Anatidae	*Anas platyrhynchos*	Mallard
11		Anatidae	*Anser domesticus*	Goose
12	Psittaciformes	Psittacidae	*Ara macao*	Scarlet Macaw
13	Columbiformes	Columbidae	*Columba palembus*	Common Wood-Pigeon
14	Gruiformes	Rallidae	*Fulica atra*	Common Coot
15	Ciconiiformes	Scolopacidae	*Calidris canutus*	Red Knot
16		Charadriidae	*Recurvirostra avosetta*	Pied Avocet
17		Laridae	*Alca torda*	Razorbill
18		Laridae	*Larus* spec.	Gull
19		Laridae	*Uria aalge*	Dovekie
20		Accipitridae	*Buteo buteo*	Common Buzzard
21		Podicipedidae	*Podiceps cristatus*	Great Crested Grebe
22		Sulidae	*Morus bassanus*	Northern Gannet
23		Phalacrocoracidae	*Phalacrocorax spec.*	Cormorant
24		Threskiornithidae	*Platalea leucorodia*	Eurasian Spoonbill
25	Passeriformes	Corvidae	*Corvus corone*	Carrion Crow
26		Fringillidae	*Passer domesticus*	House Sparrow

The skulls of the embryos were stained with alizarin alcian blue to make both bone and cartilaginous tissue visible. After staining the lower bill, tongue and larynx of the embryos were removed to simplify the measurements of the PPC. The PPC of the embryos was measured using a measuring ocular in a dissection microscope. All measurements on adult Palaeognathae, Neognathae and embryos were scaled by dividing the measurements by the width of the skull (Character A, Table 8.1.). The scaled measurements of the skulls of the embryos were used in a Principal Component Analysis to reduce the number of variables (PCA on basis of the correlation matrix with Varimax rotation). The first three principal components were tested for their descriptive value for development. If a clear relation between a principal component and development was found, the component scores of the embryos were compared to the component scores of adult Palaeognathae and Neognathae.

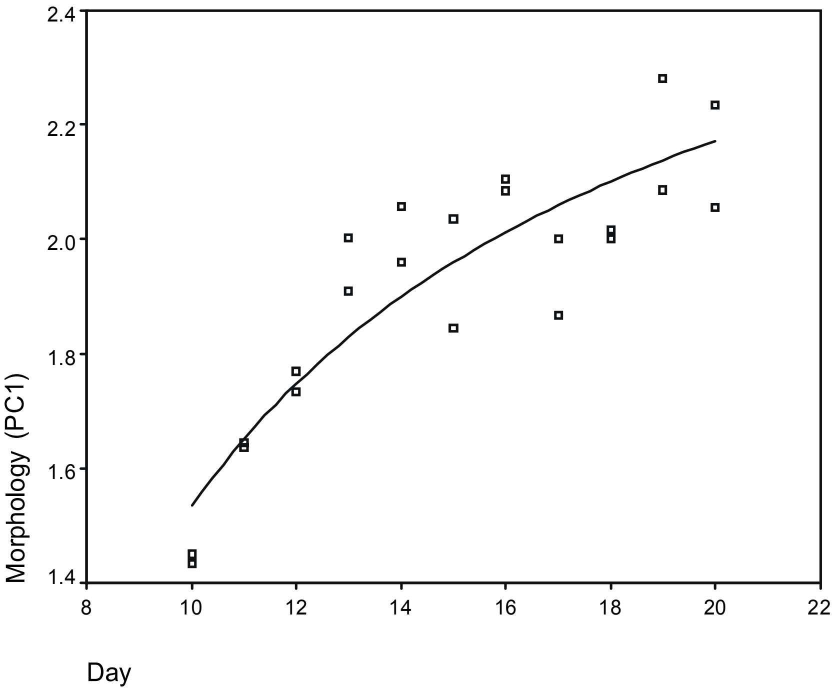

Figure 8.2. Morphology (described by the PC1) versus day of development for a neognathous bird (Gallus gallus).

Results

The first three components obtained with a Principle Component Analysis of the characters of the PPC of the developing chicken embryos, explained 70% of the total variance. The component scores of the first principle component showed a strong correlation with development (Fig. 8.2), while the scores of the second and third component did not. Therefore only the first Principle Component (PC1, variance explained: 40%) was used to describe the change in morphology during embryonic development. The characters described by the PC1 were the width of the pterygoid-palate articulation, the width of the vomer, the distance between left and right palate and the distance between the quadrates. All these characters become relatively larger during development. The loadings of PC1 for each measurement are given in table 8.1. The factor scores were used to determine the relative position of adult Palaeognathae and Neognathae on the scale determined by PC1 of the embryonic development (Fig. 8.3). Scores for Neognathae and Palaeognathae were constructed by multiplying character values and PC1 loadings from the analysis of the embryos. The position of the groups on PC1 results in a separation of the Palaeognathae and Neognathae (Fig. 8.3). Comparison of extremes

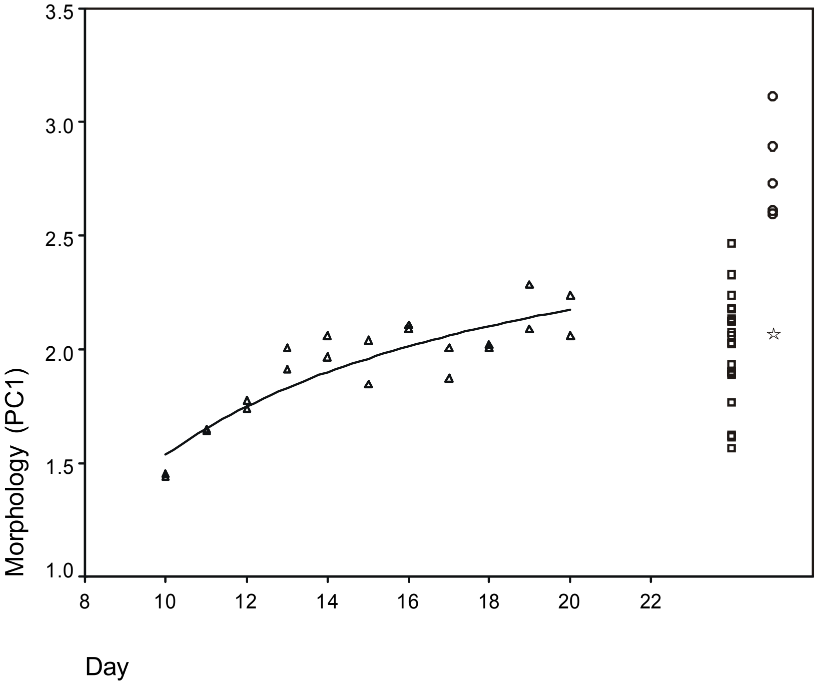

Figure 8.3. Morphology (described by the PC1) versus day of development. Far clarity adult specimens are displayed directly left of the graph. Triangles indicate embryonic neognathous birds, squares indicate adult neognathous birds, circles indicate palaeognathous birds, and the star indicate a representative of the Tinamiformes.

showed only small differences between the neognathous embryos and the adult neognathous birds. A large difference was however found between both the neognathous embryos and the Palaeognathae and the adult Neognathae and the Palaeognathae. This confirms earlier findings that the morphology of Palaeognathae and Neognathae is clearly distinct. It also shows that adult neognathous birds resemble juvenile birds more than adult palaeognathous birds.

Discussion

Using several characters of the PPC and a data-reduction method we have described a developmental curve for the chicken. This developmental curve clearly shows an increase in relative size of several characters during early development, which reaches a platform in later development. A comparison of a wide variety of adult neognathous birds and the embryos of the chicken shows that the morphology of most neognathous birds is similar to different stages of development of the chicken. Only a few adult neognathous species have a morphology index slightly higher than the oldest specimen in the developmental series of the chicken. The

Palaeognathae are significantly different from the development series and lie above the developmental curve of the chicken (except the Tinamou; Fig. 8.3) and thus above almost all neognathous species. This contradicts the hypothesis that the morphology of the Palaeognathae resembles stages of early neognathous development. These results strongly agree with the conclusions of Bock (1963), who states that the number of characters that could be juvenile in the PPC is too limited to conclude that the whole complex has evolved through neoteny. The morphological analyses that have indicated a neotenous origin of the palaeognathous PPC were both qualitative and based upon comparison (Dawson *et al.*, 1994; Jollie, 1976). Dawson claimed that neonatal thyroidectomy of songbirds results in neoteny. He found that the main character of the PPC that changed as a result of the neoteny was the reduced development of the lateral processes of the palate. Surprisingly this was interpreted as neoteny. The late development of this process might be a character in the development of songbirds, but in adult Palaeognathae the lateral process is well developed indicating that this feature of the Palaeognathae is not neotenous. Jollie (1976) concludes that the dromaeognathous (=palaeognathous) PPC represents the most primitive type of PPC, and points especially at the connection between the pterygoid and the vomer, which is clearly present in Palaeognathae. This connection, however, was never observed in the development of the chicken, although the vomer could be clearly recognised from stage 39 (day 13) onward. During development it was always small and not in contact with the pterygoid. We can therefore conclude that the findings of Dawson as well as Jollie are not a direct proof for neoteny.

In this study the development of only one species was investigated. No information is available on the variation in embryonic characters investigated across neognathous or palaeognathous species. However, under the assumption that developmental curves have the same shape in other birds and that these curves start from the same position as the chicken, it may be argued that part of the shape of the palaeognathous PPC is explained by a (relative) acceleration of the development of the characters. Note that the direction of change in the developmental series is toward the Palaeognathae but levels off, suggesting that the shape of the PPC is not simply the result of a continued growth during long incubation times of large birds. However, positive allometric growth of the whole PPC, following the same growth rules (=PC1) as in Neognathae, in combination with the size differences between Neognathae and Palaeognathae could explain part of the differences in the shape of the PPC. Alternatively, Palaeognathae may have a different starting point.

Although we have shown that the palaeognathous PPC is not neotenous, this does not necessarily show that the Palaeognathae are basal within birds (Mindell *et al.,* 1997; Mindell *et al.*, 1999; Härlid & Arnason, 1999). It still is possible that the Palaeognathae are a relatively modern order of birds, with derived characters, rather than primitive. This hypothesis is however opposed by a multitude of molecular (see Sibley & Ahlquist, 1981; Cooper *et al.*, 1992; Cooper & Penny, 1997; Cooper, 1997; Lee *et al.*, 1997), morphological data (Cracraft, 1974; Bledsoe, 1988) and behavioural data (Meise, 1963; Chapter 7). Evidence from the fossil record also points in the direction of a basal position of the Palaeognathous birds (Elzanowski, 1995;

Kurochkin, 1995). Therefore we conclude from our data, that the Palaeognathae do not have a neotenous origin, and are a basal group with derived characters within modern birds.

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