THE EVOLUTION OF THE PALAEOGNATHOUS BIRDS

Functional Morphology and Evolutionary Patterns

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Functional Morphology and Evolutionary Patterns

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Sander Wouter Sebastiaan Gussekloo
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‘Een rare boel,’, mompelde de assistant Pieps, maar de hoogleraar
wees hem scherp terecht.
‘In de wetenschap geeft het niets rares,’ vermaande hij. ‘Wij noteren
slechts, merkt u zich dat aan!’

Marten Toonder, De Mobbeweging (6825)

Contents

CHAPTER 1

INTRODUCTION

The Palaeognathae

The evolution of birds has fascinated biologist for many years. Many aspects of the evolution of birds are still uncertain, and only recently biologists seem to have reached a consensus that dinosaurs and birds are closely related (Padian & Chiappe, 1998). However, the phylogenetic relationship within birds, and the monophyly of the taxon Aves are still under dispute.

The evolution of the avian taxon Palaeognathae, especially, has puzzled biologist for more than a century. It is still unclear whether this taxon is monophyletic and what the phylogenetic relation is between this taxon and all other birds.

In this thesis functional and evolutionary morphology is used to investigate the evolution of the Palaeognathous birds with special focus on cranial characters and their interrelation with feeding behaviour.

The species

Following the systematics of Sibley and Monroe (1990), the taxon Palaeognathae consists of a very limited number of extant species. The taxon Palaeognathae is divided into two subtaxa, the Ratites and the Tinamous. The Ratite taxon consists of only 10 species: the greater and smaller Rhea (*Rhea americana*, *Pterocnemia pennata*) from South-America, the Ostrich (*Struthio camelus*) from Africa, three species of cassowaries from New-Guinea and northern Australia (*Casuarius casuarius*, *C. bennetti*, *C. unappendiculatus*), the Emu (*Dromaius novaehollandiae*) from Australia and three species of Kiwis (*Apteryx australis*, *A. owenii*, *A.haastii*) from New-Zealand. The second sub-taxon is represented by approximately 50 species of Tinamous in 9 genera (*Tinamus*, *Nothocercus*, *Crypturellus*, *Rhynchotus*, *Nothoprocta*, *Nothura*, *Taoniscus*, *Eudromia*, *Tinamotis*) all from South-America. All the Ratites are flightless, while the Tinamous are all poor flyers.

In addition to the extant species a number of fossil groups is also considered to belong to the Palaeognathae. The two best-known fossil palaeognathous groups are the Aepyornithidae or Elephant-birds of Madagascar (Rich, 1979, 1980) and the Dinornithidae or Moas from New-Zealand (Owen, 1840; Archey, 1941; Oliver, 1949; Cracraft, 1976; Millener, 1982; Worthy, 1988a,b; 1989). Another group of Palaeognathae, found in Europe and North America, are the Lithornithidae (Houde, 1988). Finally, some incidental findings have been considered to be Palaeognathae also, but their taxonomical position is still uncertain. (Ambiortidae: Kurochkin, 1982; Eleutherornithidae: Harrison & Walker, 1979; Houde & Haubold, 1987; Gansuidea: Hou & Lui, 1984; Opisthodactylidae: Alvarenga, 1983; Palaeocursornithidae: Kessler & Jurcsak, 1984; Bock & Bühler, 1996; Remiornithidae: Lemoine, 1881; Lydekker, 1891; Martin, 1992 Patagopterygidae: Alvarenga & Bonaparte, 1992, but see also Chiappe & Calvo, 1989; Chiappe 1990, 1991). For the phylogeny of these fossil bird groups I refer to the work of Kurochkin (1995).

Morphological characters

The first who made a distinction between the Palaeognathae and all other birds was Merrem (1813). His distinction was mainly based on the keelless sternum of the Ratites. Since then various morphological characters have been used to describe the differences between the Palaeognathae and all other birds. Many of the characters proved not to be completely distinctive and only a few characters remain.

The first character that is considered to be specific for the Palaeognathae is the unfused condition of the pelvis. This character was first noted by Pycraft (1900) and is described as the open ilioischiatic fenestra. The fenestra is called open because the *ilium* and the *ischium* are not fused as in neognathous birds. The second character is the apparent segmentation of the rhamphotheca as described by Parkes and Clark (1966). They describe very distinct grooves in the rhamphotheca that separate the medial nail-like section from the lateral parts. The third character is the morphology of the ear, described by several derived characters (Starck, 1995).

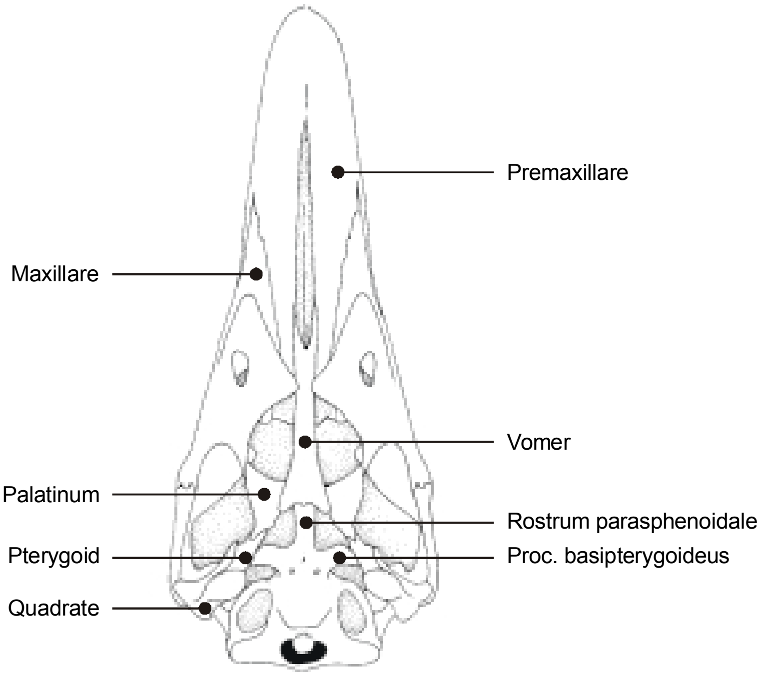

Figure 1.1. Ventral view of the palaeognathous Pterygoid-Palatinum Complex (PPC) in the Greater Rhea (Rhea americana).

The fourth character is the presence of the *Musculus geniohyoideus* (Müller and Weber, 1998), which is present in reptiles and the Palaeognathae only. Finally, the most important character is probably the palaeognathous palate, first recognised by Huxley (1867). Although always referred to as the palaeognathous or dromaeognathous palate, the character is a combination of characters found in the bony elements of the ventral aspect of the facial part of the skull. These elements include the quadrates, (*Os quadratum*), the pterygoids (*Os pterygoideum*), the palates (*Os palatinum*), and the vomer (*Vomer*). This combination of the pterygoids, palates and vomer ('the palate') will be referred to as the Pterygoid-Palatinum Complex (PPC).

The existence of the palaeognathous PPC has often been discussed. The first complete description of the PPC's of all the Palaeognathae was given by McDowell (1948). Based on the

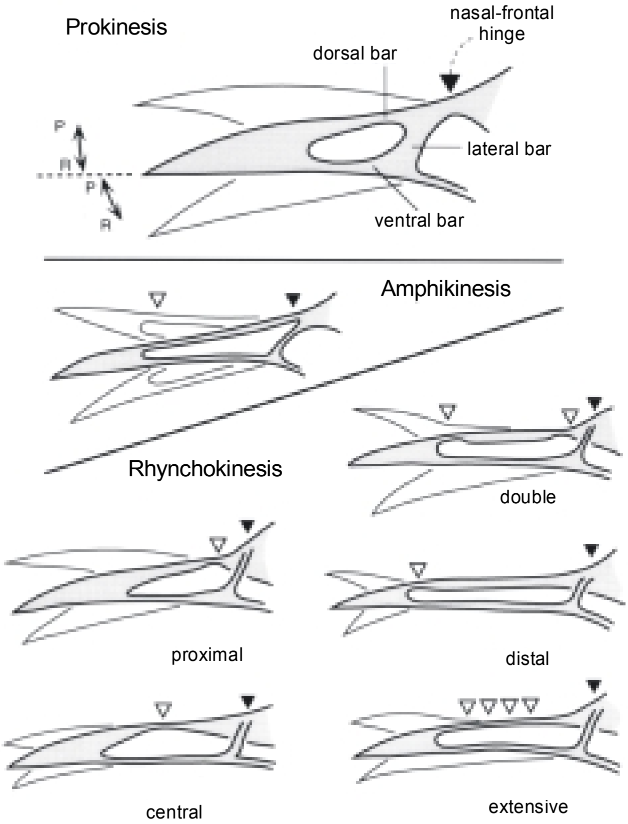

Figure 1.2.Types of cranial kinesis in birds (Adapted from Zusi, 1984). P=protraction, R=retraction; solid pointers indicate the nasal-frontal hinge; open pointers indicate additional bending zones in the dorsal bar.

large variation in the morphology of these PPC's he concluded that a uniform palaeognathous PPC does not exist and that the palaeognathous PPC was therefore not a true character. This was later opposed by Bock (1963), who stated that a uniform palaeognathous PPC could be described and that it was a character found in the Palaeognathae only. He described the palaeognathous PPC with the following characters (Fig. 1.1; Bock 1963, p. 50): a) the vomer is relatively large and articulates with the premaxillae and the maxillo-palatines anteriorly and (except for the Ostrich) with the pterygoids posteriorly; b) the pterygoid prevents the palatine from articulating with the *Rostrum parasphenoidale*; c) the palatine connects to the pterygoid along a suture; d) the basitemporal articulation is large, and is found near the posterior end of the pterygoid; e) the articulation between the pterygoid and the quadrate is complex, and includes part of the orbital process of the quadrate. He concluded that the palaeognathous palate as a whole presents a general configuration similar in all birds possessing it, and sharply distinct from the condition found in all other birds.

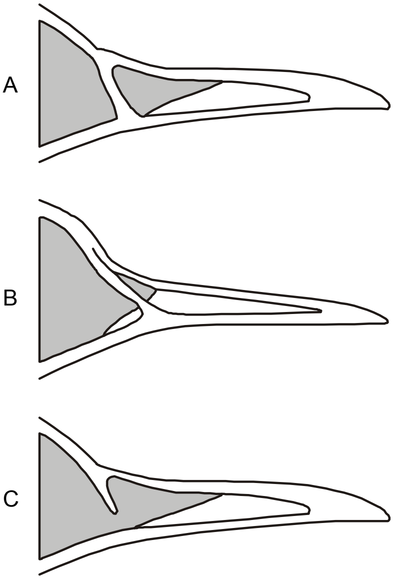

Figure 1.3. Different types of nostrils. Grey areas indicate inter-orbital and inter-nasal septa.

A. Neognathous holorhinal
B. Neognathous schizorhinal
C. Palaeognathous holorhinal

The function of the PPC has been described for neognathous birds only (Bock, 1964). The PPC plays an important role in the movement of the upper bill. The movement of the upper bill is induced by a rotation of the quadrate. The rotation is transferred into a rostral/caudal movement of the pterygoid and palate. The rostral/caudal movement of the palate results in a rostral/caudal movement of the lower part of the upper bill, which results in a rotation around a hinge or flexible zone in the upper bill. Based on the position of the flexible zones three main types of kinesis are distinguished (Fig. 1.2; Zusi, 1984). The first and the most common type is prokinesis. In this type the upper bill consists of three rigid bones, which do not move relative to each other. The upper bill moves as a whole around the nasal-frontal hinge. In the second type, rhynchokinesis, bending occurs within the upper bill through bending zones in both the dorsal and ventral part. The third type, amphikinesis can be considered a combination of prokinesis and rhynchokinesis. Rhynchokinesis itself can be divided into five subtypes based on the number and position of the bending zones in the upper bill. Three types have a clear narrow bending zone and are named after the position of the bending zone in the upper bill: proximal rhynchokinesis, distal rhynchokinesis, and central kinesis. In double rhynchokinetic bills two bending zones are present: one at the proximal and one at the distal end. Finally, extensive rhynchokinesis is characterised by an elongated bending area along the central area of the upper bill.

To make rhynchokinesis possible, not only flexible zones must be present in the upper bill, but the movement inducing the rotation must also be transferred by the non-rotating proximal part of the upper bill, to the rotating distal part. This can be achieved by a uncoupling of the movement of the dorsal and ventral bony bars of the upper bill. Uncoupling allows the ventral bar to slide forward while the dorsal bar remains stationary. This is achieved in two different ways in avian evolution. The first and most common solution is the development of a special type of nostril, the schizorhinal nostril (Fig. 1.3). In prokinetic birds the dorsal and ventral bars are connected at the caudal side through a rigid lateral bar that has its dorsal end rostral to the nasal-frontal hinge. To uncouple the movement of the dorsal and ventral bar, this lateral bar becomes flexible and its dorsal connection shifts caudally to the area behind the nasal-frontal hinge. This change improves bending in the lateral bar and therefore the uncoupling of the dorsal and ventral bar. In the second more rigorous solution the lateral bar is broken, or more accurately, reduced to a small highly flexible ligament. The position of the dorsal end of the lateral bar, however, is still positioned in front of the nasal-frontal area, and the nostril is therefore holorhinal (Fig. 1.3).

All prokinetic Neognathae have a holorhinal nostril. All rhynchokinetic Neognathae possess a schizorhinal nostril. The Palaeognathae are the only living birds that possess central rhynchokinesis according to the definition of Zusi (1984) and achieved uncoupling of the dorsal and ventral bar with a holorhinal nostril and a ligamentous lateral bar. As mentioned earlier the Palaeognathae also have a special configuration of the PPC. Since this combination is only found in the Palaeognathae several authors have tried to connect these three characters (Hofer 1954, Simonetta, 1960, Bock 1963) by a functional explanation. The validity of the proposed functions will be discussed later in this thesis.

Phylogeny and evolution of the Palaeognathae

An important question has always been whether the Palaeognathae are a monophyletic taxon or not. Based on the morphology of the PPC Huxley (1867) considered the Palaeognathae monophyletic. This was later confirmed by characters of the axial skeleton (Mivart, 1877). Fürbringer (1888) proposed that the large diversity in the morphology of the palaeognathous palate indicated independent origins of the different Palaeognathae and that they were therefore polyphyletic. The discussion about the monophyly of the Palaeognathae lasted until methods came available that were not directly dependent on morphological differences. From 1960 onward (Sibley, 1960) several molecular techniques were used to test the monophyly of the Palaeognathae. These studies seem to have solved the dispute since they all indicate that the Palaeognathae are monophyletic (e.g. Sibley & Ahlquist, 1990; Cooper *et al.*, 1992; Cooper, 1994; Caspers *et al.*, 1994; Lee *et al.*, 1997; Cooper & Penny, 1997; Cooper, 1997).

The origin of the Palaeognathae, however, has never been solved and several hypotheses about the evolution of this taxon have been postulated. The first and most generally accepted one states that the Palaeognathae are the most basal group within modern birds (Fig. 1.4a; Feduccia, 1995). This is confirmed by a large number of molecular analyses (e.g. Sibley & Ahlquist, 1990; Cooper *et al.*, 1992; Cooper, 1994; Caspers *et al.*, 1994; Lee *et al.*, 1997; Cooper & Penny, 1997; Cooper, 1997; van Tuinen *et al.*, 1998; Groth & Barrowclough, 1999) and morphological analyses including extinct taxa (Elzanowski, 1995; Kurochkin, 1995). The alternative theory states that the Palaeognathae are a non-basal group and that the characters that are presumed to be primitive have evolved through neoteny (Fig. 1.4b; de Beer, 1956; Jollie, 1976). This hypothesis is supported by some recent molecular phylogenetic analyses (Mindell *et al.*, 1997; Mindell *et al.*, 1999; Härlid & Arnason, 1999) and experiments in which palaeognathous characters were found in neognathous songbirds after neonatal thyroidectomy (Dawson *et al.*, 1994). Based on the present knowledge it is very difficult to decide which of the two hypotheses is correct. At present only the argument that a majority of studies indicate a basal position of the Palaeognathae within birds can be used to prefer that hypothesis to the hypothesis that the Palaeognathae have a neotenous origin.

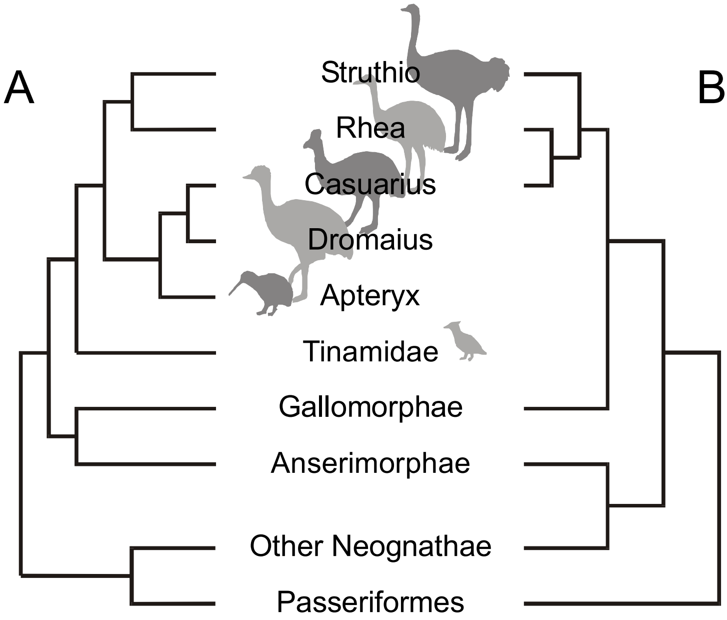

Figure 1.4. Alternative avian phylogenies. A. Palaeognathae at a basal position (DNA-DNA hybridization; Sibley & Ahlquist, 1990). B. Palaeognathae at a derived position (DNA-sequencing; Härlid et al., 1998).

Avian evolution

Although several hypotheses exist about the origin of birds, the most generally accepted hypothesis states that the birds have evolved from a Theropod dinosaur (Padian & Chiappe, 1998; Sereno, 1999). The origin of birds is more precisely situated within the Maniraptora, a taxon within the Coelurosauria. Within the Maniraptora birds are probably closely related to the Dromaeosauridae. Other taxa within the Maniraptora are the Troodontidae and the Oviraptorosauridae (Sereno, 1999). The origin of the earliest birds is estimated to have been in the Late Jurassic (150 MYA), while orders of modern birds are thought to have originated in the Mid-Cretaceous (100 MYA). At the transition of the Cretaceous to the Tertiary (65 MYA) a mass-extinction took place in which the majority of the dinosaurs became extinct. The only groups from the taxon Dinosauria that were able to pass the Cretaceous-Tertiary (K-T) boundary were representatives of the extant avian orders, which showed a large radiation during the Tertiary.

Two different hypotheses have been postulated about the survival of the extant bird orders across the K-T boundary. The first and most generally accepted hypothesis, states that about 20 modern avian orders were present in the Cretaceous and that all passed the K-T boundary without severe extinction (Chiappe 1995; Cracraft, 1986; Sibley and Ahlquist, 1990). In contrast to this hypothesis Feduccia (1995) states that a large number of bird-orders was present in the Cretaceous, which almost all became extinct during the Cretaceous-Tertiary transition. Of all modern orders only one type was assumed already present in the Late Cretaceous. Feduccia describes this bird as a ‘Transitional shorebird’. Feduccia states that from all bird orders present in the Late Cretaceous only this ‘Transitional shorebird’ survived the mass-extinction during the Cretaceous-Tertiary boundary. Subsequently this ‘Transitional-shorebird’ gave rise to all modern avian taxa.

Functional (evolutionary) morphology offers a good framework to solve the conflict between the ‘single bird survivor’ and the ‘multiple bird survivor’ theory. Because wide-scale extinction took place both within and between taxa, it is clear that the change in environment in the period of the K-T transition resulted in high selection pressures. Organisms that were sufficiently adapted to the new environment were able to pass the K-T boundary, while others were not. In other words, the environment put high functional demands on the morphology of all organisms.

It is possible to determine the specific functional demands that were acting on the avian morphology during the K-T transition. These functional demands can be combined with the known morphology of the fossil bird groups from the Cretaceous, and for each group it can be tested whether it fits the functional demands. Only when the morphology is adapted to the functional demands the bird group is expected to survive. This method makes it possible to determine whether only the ‘Transitional Shorebird’ of Feduccia was able to survive the K-T transition or that other groups of birds were able to pass the transition as well.

This method was developed and used by Zweers *et al.* (1997; see also Zweers & Vanden Berge, 1997b) to describe a possible avian evolutionary pathway. Their analysis focused only on cranial morphology in relation to the trophic system of birds and cannot be unconditionally extrapolated to the entire organism. According to Zweers *et al.* (1997) the available food during the K-T transition can be divided into five different groups: 1) aquatic invertebrates and fish, 2) burrowed invertebrates, such as crabs, worms and molluscs in coastal mudflats 3) aquatic plants, including seaweeds 4) tough foliages, such as grasses, supplemented by seeds, insects and possibly small vertebrates and 5) predators on the first four groups. Based on these food-types the authors hypothesise that dinosaurs needed large biting forces, a highly modifiable feeding apparatus and a wear-resistant keratin-layer on the jaws. The extra biting force is needed to process the often tough food-items; the wear-resistant keratin-layer is needed since these food-items may damage the beaks, and keratin is easy to replace. The keratin-layer is also highly adaptable and special features such as holding ridges can easily evolve in keratin.

According to Zweers *et al.* (1997) these functional demands are fulfilled by two adaptations of the skull. The first is the evolution of a keratin-layer along the beak, known as the rhamphotheca. The second is a detached palate in combination with upper bill kinesis. In their hypothesis this uncoupling of the palate results in an extra contribution of the pterygoid muscles to the biting forces through the moveable upper bill. This detachment of the palate resulted in three different types of moveable upper bills: 1) a meso-/pre-kinetic lineage including *Archaeopteryx*, 2) a central rhynchokinetic lineage including the Palaeognathae and 3) a prokinetic lineage including the Neognathae. Since only birds had a moveable upper bill in combination with a rhamphotheca they were able to survive the K-T boundary. It was suggested that not just a single “Transitional shorebird’ survived the K-T Transition (Feduccia, 1995) but four different trophic types of birds. These bird-types are a browser type (Palaeognathae lineage) and three other trophic types from the neognathous lineage (grebe-like catcher, plover-like prober and rail-like slicers).

In this hypothesis especially the position of the Palaeognathae is remarkable since they are conventionally considered the most basal group within modern birds and the sister group of the Neognathae. In this thesis an analysis is made of the feeding apparatus of the Palaeognathae in order to test the hypotheses of Zweers *et al.* (1997) that a) the morphology of the palaeognathous skull results in additional biting forces and b) the Palaeognathae and Neognathae are phylogenetically not closely related. The Palaeognathae are especially of interest since their morphology of the PPC, the element that plays the key-role in the hypothesis of Zweers *et al.* (1997), is so remarkably different.

To study the evolutionary significance of the palaeognathous PPC a number of questions must be answered. First the question will be addressed whether the palaeognathous PPC can be defined and whether it is found within the Palaeognathae only. Using an outgroup comparison method it will be investigated whether the palaeognathous PPC represents a primitive or derived condition within birds. After the presence of the character has been established it is necessary to determine its role and function, and the effect of this function on the morphology. In neognathous birds the PPC is important in the movement of the upper bill. Because the Palaeognathae possess both a unique PPC morphology and a unique type of kinesis (central rhynchokinesis) it must be tested whether the type of kinesis has an effect on the PPC morphology. The PPC morphology of a neognathous rhynchokinetic species will be analysed and characters specific to either rhynchokinesis or to the feeding behaviour will be described. In addition the feeding behaviour of the neognathous rhynchokinetic bird will be described in order to determine the advantage of rhynchokinesis and to determine when maximal rhynchokinesis occurs. These characteristics of rhynchokinetic feeding behaviour can then be used to test whether the morphology of the palaeognathous skull is adapted to rhynchokinesis and whether it serves the same function in Palaeognathae as in Neognathae. This is tested by determining the displacement patterns of the bony elements in the skull during upper bill elevation. To measure these displacements a new roentgen-stereophotogrammetry method is developed, which makes it possible to determine the displacement in three dimensions with an accuracy of 0.12mm. The displacement of bony elements in the skull during upper bill elevation was determined in three palaeognathous species, and for comparison in a rhynchokinetic and a prokinetic neognathous bird. The palaeognathous skull was further investigated for the presence of characters specific for rhynchokinesis and a configuration of the skull that is optimal for rhynchokinetic feeding behaviour. The feeding behaviour of the Palaeognathae is analysed to investigate the role of rhynchokinesis and to determine which functional demands have resulted in the specific palaeognathous PPC morphology. Finally, it is tested whether the palaeognathous PPC might have evolved through neoteny. This is done by a comparison of adult palaeognathous skulls, with the skulls of a neognathous bird in several stages of development. The results of these studies will be used to determine which selective forces have resulted in the specific morphology of the palaeognathous skull.

CHAPTER 2

THE PALAEOGNATHOUS PTERYGOID-PALATINUM COMPLEX. A TRUE CHARACTER?

Summary

Molecular analyses show that modern birds can be divided into two major taxa, the Palaeognathae and the Neognathae. This division was already proposed by Merrem in 1813, based on morphological characters. One of the most prominent discriminating characters is the morphology of the Pterygoid-Palatinum Complex (PPC), which is different in palaeognathous and neognathous birds. There are very few other morphological characters that support this division and even the differences in PPC have been under dispute. A discriminant analysis based on quantitative measurements of the PPC shows that a large difference between the two morphologies exists, and that the Tinamidae possess an intermediate form. An evolutionary maximum-likelihood analysis suggests that the PPC of the Palaeognathae is more primitive than that of the Neognathae. A functional interpretation of the differences in the PPC between the Palaeognathae and the Neognathae indicates that the palaeognathous PPC is not, as generally accepted, an adaptation related to rhynchokinesis, but probably contributes to reinforcement of the skull after the loss of both the postorbital and nasal bar.

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Introduction

Ever since Merrem (1813) divided birds into two groups, the Carinatae and the Ratitae, the latter group has been the source of many disputes. The Ratitae, later grouped with the Tinamous in the superorder Palaeognathae by Pycraft (1900), consists of ten living species (Ostrich, *Struthio camelus*; Rheas, *Rhea americana*, *Pterocnemia pennata*; Cassowaries, *Casuarius casuarius*, *C. bennetti*, *C. unappendiculatus*; Emu, *Dromaius novaehollandiae*; Kiwis, *Apteryx australis*, *Apteryx owenii*, *Apteryx haastii*) and a number of extinct taxa, such as Moas (Oliver, 1949, Cracraft 1976) and Elephantbirds (Cracraft, 1974). The Tinamous comprise approximately 50 living species from Latin America. The systematic position of the Tinamous is uncertain. Several authors place the Tinamous with the Ratites (Cracraft 1974, Sibley & Ahlquist 1990), while others consider them to be neognathous (Gingerich, 1976). The dispute around the division between neognathous and palaeognathous birds includes the existence of the group as a systematic or phylogenetic entity, the monophyly of the group, and the question whether this group is primitive or derived within birds. The first issue is of course essential and the specific characters that discriminate the Palaeognathae from all other birds are of great importance. Although a large number of recent molecular studies has indicated that the Palaeognathae are a single monophyletic taxon (Sibley & Ahlquist, 1990; Cooper *et al.*, 1992; Cooper, 1994; Caspers *et al.*, 1994; Lee *et al.*, 1997; Cooper, 1997) only a few morphological characters have been described that are typical for the Palaeognathae. These specific morphological characters are the palaeognathous palate (=dromaeognathous palate), first described by Huxley (1867), the unfused condition of the pelvis, first described by Pycraft (1900), the apparent segmentation of the rhamphotheca described by Parkes & Clark (1966), and the presence of the *Musculus geniohyoideus* in the palaeognathous lingual apparatus (Müller & Weber, 1998). The most prominent character in the discussion about the Palaeognathae has always been the palaeognathous palate. Since this term does not fully describe the morphology, it will be further referred to as the palaeognathous Pterygoid-Palatinum Complex (palaeognathous PPC).

Huxley (1867) found that in the palaeognathous PPC the caudal ends of the palatines and the rostral ends of the pterygoids do not articulate with the *Rostrum parasphenoidale* and that there is a strong *Processus basipterygoideus*. McDowell (1948) was the first to make a thorough osteological analysis of the palaeognathous palate. He concluded that a palaeognathous PPC cannot be defined, because of the large variation in morphology within the Palaeognathae, and the presence of some of the 'palaeognathous' characters in neognathous birds. This was later disputed by Bock (1963), who claimed that the palaeognathous PPC as a whole can be distinguished from the neognathous PPC. As he puts it: 'The palaeognathous palate as a whole presents a general configuration similar in all birds possessing it, and sharply distinct from the condition in all other birds'. The characters used by Bock to describe this condition include the shape of the vomer, the pterygoid-palate articulation and in its relation to the *Rostrum paraspenoidale*, the articulation with the *Processus basipterygoideus* and the pterygoid-quadrate articulation. Bock was also one of the few authors who gave a functional interpretation of the palaeognathous PPC. All functional interpretations relate the morphology of the complex to rhynchokinesis (Hofer, 1954; Simonetta 1960; Bock, 1963). In this type of cranial kinesis only a small rostral part of the upper bill can be elevated (Bock, 1964; Zusi, 1984). Hofer (1954) considers the palaeognathous PPC similar to the desmognathous condition as found in Anseriformes (Huxley, 1867; de Beer, 1937). The desmognathous condition is never found in combination with rhynchokinesis outside the Palaeognathae. Hofer considers the special morphology of the palaeognathous PPC as a condition for the combination of a holorhinal nostril and rhynchokinesis. Holorhinal nostrils are characterised by bony external nares whose concave caudal borders lie rostral to the caudal end of the nasal process of the premaxillae. In neognathous birds that show rhynchokinesis a schizorhinal nostril is present, which is characterised by a slit-like caudal border, situated caudal to the end of the nasal process of the premaxilla. Since in all rhynchokinetic birds the ventral part of the upper bill must move forward relative to the dorsal part, an uncoupling of dorsal and ventral bars is necessary. While schizorhinal nostrils uncouple the dorsal and ventral bars, a bony bridge connects these bars when holorhinal nostrils are present. Bock (1963) follows Hofer in saying that rhynchokinesis is always observed in combination with a schizorhinal nostril, but that in the rhynchokinetic Palaeognathae the nostril can be described as holorhinal. When the nostril is holorhinal uncoupling can be accomplished by a gap in the nasal bone, as found in Palaeognathae. The more rigid structure of the palaeognathous PPC is explained by Bock as an adaptation to the rigid dorsal bar of the upper bill. This bar is relatively thick and large forces are necessary to bend it. To ensure efficient transfer of force the elements of the PPC are strong and rigid, and placed in a straight line as observed in the Palaeognathae (Bock, 1963, p. 48).

To test the hypothesis that the morphology of the PPC within the Palaeognathae is different from that of all other birds, several quantitative characters of skulls of 26 extant bird species were taken. A discriminant analysis is used to test whether these characters allow a complete separation between Palaeognathae and Neognathae. The PPC characters are also used for a comparative analysis. An outgroup is used to determine possible evolutionary patterns, which might indicate whether the Palaeognathae are primitive or derived within modern birds. Functional implications of the differences in characters will be formulated, and their consequences for a connection between a palaeognathous PPC and rhynchokinesis are discussed.

Materials and Methods

Taxonomical names of all bird species and families are according to the classification of Sibley and Monroe (1990, 1993). For the analysis 26 species of the Class Aves and one species of the Class Reptilia were used. The avian species were taken from 9 orders, 18 families, and 26 genera. The species of the Class Reptilia (order Crocodillia) was used as outgroup for the phylogenetic analysis. This taxon was chosen as a near living relative of all birds (Hedges & Poling, 1999). No fossil birds or dinosauria were used for this analysis due to the lack of good fossil material of the PPC. All 26 avian species and the outgroup are summarised in table 2.1.

For the similarity analysis of the PPC 17 descriptive characters and one standard measure were taken of all 27 specimens. The characters were distributed over the whole PPC and are summarised in table 2.2 and figure 2.1. Anatomical nomenclature is according to Baumel *et al.* (1993).

Not all characters are present in all specimens; especially the vomer is highly variable and is reduced in many species. When a character is totally absent the measure of the character was determined to be zero. Characters were measured using an electronic calliper rule (Sylvac, accuracy 0.01 mm). Each measurement was taken twice and the average was used for further calculations. Differences between repeated measurements did not exceed 0.1 mm. To eliminate size effects, all measurements were standardised by dividing them by the value of a standard character A (skull width, see table 2.2).

A stepwise discriminant analysis was performed to determine the discriminating characters between the palaeognathous PPC configuration, the neognathous PPC configuration and the configuration in the outgroup. In a discriminant analysis each individual is appointed to a group *a priori*, in this case either to the Palaeognathae or the Neognathae. Based on that division two

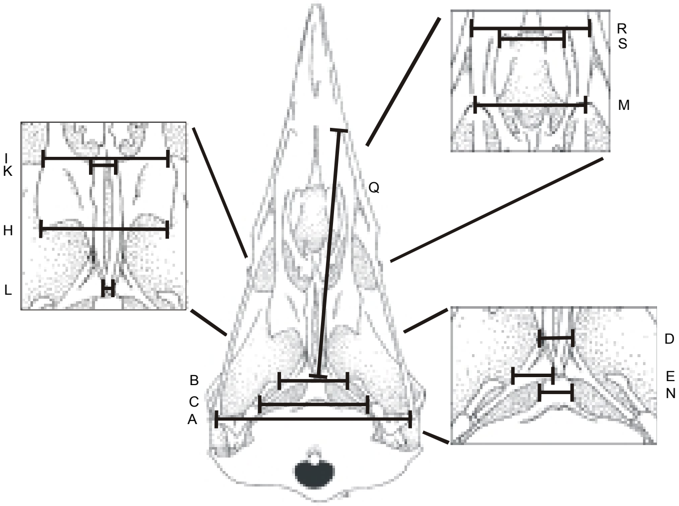

Figure 2.1. Skull of the crow (Corvus corone) in ventral view. Inserts are enlargements of areas indicated by the lines. Letters refer to characters in table 2.1. The characters of the vomer cannot be represented since the vomer is reduced in this species. Other characters omitted from this figure for 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 2.1. Species used in distance analysis. Names according to the classification of Sibley and Monroe (1990,1993).

No	Order	Family	Species	Common name
0		Alligatoridae	*Caiman* spec.	Cayman
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-Pigeor
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

canonical discriminant functions are calculated, which describe the maximum separation between the two groups. Since the exact position of the Tinamidae is not known, the Tinamid-species was not appointed to any group *a priori*. The discriminant functions are used by the procedure to assign each individual to either the Palaeognathae or Neognathae independent of their *a priori* group membership. When the discriminant functions completely separate the groups, the *a priori* group-membership is the same as the membership determined from the discriminant functions (Manly, 1994).

An evolutionary tree was estimated using a Continuous Characters Maximum Likelihood Method (Felsenstein, 1981, 1993). The tree is rooted by the Cayman and is assumed to represent the pathways in PPC morphology evolution. Within the Maximum Likelihood method the options ‘Global rearrangements’ was used to optimise the tree. Species were added at random to the tree, and this random procedure was repeated a thousand times to find the optimum tree from all these runs. The Cayman was appointed outgroup as closest living relative of all birds. As comparison for the Maximum likelihood analyses, a phylogenetic tree based on DNA-DNA hybridisation was used. The data for this tree were obtained from Sibley and Monroe (1990, 1993). Species not in the DNA-DNA hybridisation tree were placed at the position of a closely related species.

Table 2.2. Characters used for distance analysis.

-	**Character**
A	Skull width at the quadrate-jugal articulation [standard]
B	Distance between most distal points of *Processi Orbitalis Quadrati*
C	Width at pterygoids at quadrate-pterygoid articulation
D	Width of pterygoids at pterygoid-palate articulation
E	Maximal width of the right pterygoid in the transversal plane
F	Width of the vomer [caudal]
G	Width of the vomer [rostral]
H	Width of the caudal part of the palatal wings (pars lateralis]
I	Maximal width of the palate at the medial ending of pars lateralis
K	Width between palates at position ‘I’
L	Width of palate at pterygoid-palate articulation
M	Internal width at the jugal-premaxilla articulation
N	Width of the *R. parasphenoidale* incl. *P. basipterygoidei* if present
O	Distance *Foramen magnum* to measurement ‘N’
P	Distance *Foramen magnum* to medial fusion of bony elements
Q	Maximal length palate
R	Width at palate-premaxilla articulation
S	Internal width at palate-premaxilla articulation

Results

PPC characters discriminating between Palaeognathae, Neognathae and the outgroup

The results of the discriminant analysis show that the measured characters can define very accurately the difference between Palaeognathae, Neognathae and the outgroup. Two canonical discriminant functions are determined by the analysis, each with its own discriminating meaning. The first function describes the differences between Neognathae and Palaeognathae, while the second function describes mainly the differences between the outgroup and all birds. The discriminating characters and their relative importance in the discriminating functions are given in table 2.3. The first discriminating function describes seven characters important in the discrimination between the Palaeognathae and the Neognathae. These seven characters can be combined to the following description of the palaeognathous PPC: the *Processus basipterygoideus* is relatively large, the *Processi orbitalis quadrati* are

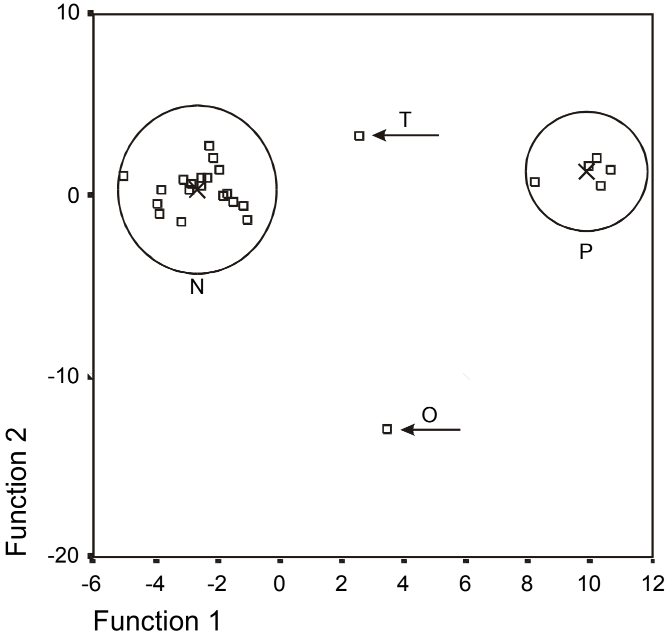

Figure 2.2. Discriminant plot. The X-axis represent the axis of maximal differentiation, the Y-axis of second maximal differentiation. N=Neognathae, P=Palaeognathae, T=Tinamidae, O=Outgroup. Crosses indicate the centre of each group.

relatively small, and the Pterygoid-Palate articulation, the vomer, and the pterygoids are all broader in Palaeognathae (Fig. 1.1). The distance between the *Foramen magnum* and the medial fusion of the Pterygoid/Palate is larger in Palaeognathae. Figure 2.2 shows the distribution of the species when the two discriminant functions are plotted against each other. It is clear that the functions can be used to distinguish between the different groups. When the results of the discriminant analysis are used to determine the position of the specimens without an *a priori* group membership a 100% correct placement is obtained. Since the Tinamou was not appointed an *a priori* group membership, its placement could not be tested. The Tinamou is placed almost exactly in between the Palaeognathae and the Neognathae. Using the discriminant functions the Tinamou is calculated to be neognathous based on the somewhat smaller distance to the Neognathae than to the Palaeognathae.

Evolutionary Morphological Clustering

A total of 22775 different trees were analysed; the tree with the highest likelihood is given in figure 2.3. The logarithmic likelihood of this tree is 836.89. This unrooted tree shows clearly that the Palaeognathae are clustered together and are more closely related to the outgroup than all other birds. When considering these characters for their taxonomical value the tree shows that

Table 2.3. Discriminating characters between palaeognathous and neognathous PPC's.

	Variable	**Function 1**	**Function 2**
N	Width of the *R. parasphenoidale* incl. *P. basipterygoidei* if present	1.249	0.463
B	Distance between most distal points of *Processi orbitalis quadrati*	1.025	0.370
L	Width of palate at pterygoid-palate articulation	0.851	-0.118
P	Distance *Foramen magnum* to medial fusion of bony elements	-0.770	0.804
F	Width of the vomer [caudal]	0.656	0.169
E	Maximal width of the right pterygoid in the transversal plane	0.442	-0.822
D	Width of pterygoids at pterygoid-palate articulation	-0.094	-0.804

members of the same family (based on molecular data) are always clustered closely together. Only the members of the family Laridae are situated at different branches, but the total distance between the members is small. At a higher taxonomical level the clustering based on PPC-characters does not follow the molecular clustering completely. In almost all cases the members of the ordines with multiple species in the analysis (Struthioniformes, Galliformes, Anseriformes, Passeriformes and Ciconiiformes) are clustered together. The species of the order with the largest number of specimens (Ciconiiformes) are all represented in one large cluster. Only the Coot, Crow and Galliformes can be considered 'misplaced' within this group. The three Anseriformes are also represented by one cluster. The position of the Ara, as a sister group of the Screamer (*Anhima*), is probably due to 'long branch attraction' (Hendy & Penny, 1989). Long branch attraction is an effect of parsimony methods, which tends to cluster specimens with long evolutionary branches. The specimens of the Passeriformes are placed at relatively large distances from each other. However, no intermediates were present in this analysis, which might, in combination with 'long branch attraction', result in the different placement.

Discussion

From the analysis it is clear that the PPC of the Palaeognathae is completely different from that of neognathous birds and that a uniform palaeognathous PPC can be described. Although the morphology of the PPC of the Tinamidae seems to be intermediate between the Ratites and the neognathous birds it is clearly distinct from neognathous birds. In this study the single species representing the Tinamidae was grouped together with the Neognathae but this was based on a very small difference in distance. This makes the exact position of the Tinamidae unclear, but other independent morphological characters (Pycraft, 1900; Parkes & Clark, 1966, Müller & Weber, 1998) and molecular data (Sibley & Ahlquist, 1990; Cooper *et al.*, 1992; Cooper, 1994; Caspers *et al.*, 1994; Lee *et al.*, 1997; Cooper, 1997) show that the Tinamous are palaeognathous.

The characters found in this analysis are all quantitative measurements and can therefore not be used to test all the characters of the palaeognathous PPC as given by Bock (1963), who also included qualitative characters. It is however clear that the discriminating characters found in this analysis are similar to those reported by Bock. The main differences between the

Palaeognathae and Neognathae found in this study are 1) a large *Processus basipterygoideus,* 2) relatively short *Processi orbitalis quadrati*, 3) a broad articulation between the pterygoid and palatine bones, 4) the articulation between pterygoids and palates is relatively rostrally situated, 5) the vomer is broad and 6) the pterygoids are well developed in a medio-lateral plane.

The discriminant analysis and the maximum-likelihood method show similar results for the difference between the Ratites, the neognathous birds and the intermediate position of the Tinamous. Based on other characters the Tinamous are considered to be palaeognathous. In our study the Tinamous are represented by a single species only, which is neither the most primitive nor the most derived species of all Tinamidae (Sibley and Monroe, 1990). When adding more species of the Tinamidae, especially the more primitive ones, the calculated position of the Tinamidae may shift toward a more Ratite position on the first discriminant function confirming a Ratite classification for the Tinamidae.

The demonstration of a typical palaeognathous PPC indicates a monophyly of the Ratites. The position of the Tinamous remains uncertain but it is clear that the Tinamidae are the closest relatives of the Ratites. Although the morphology of the PPC of the Tinamous does not fit closely in the definition, it is still very distinct form the neognathous condition. We therefore consider the PPC of the Tinamous also Palaeognathous.

When we assume that the Cayman outgroup is a good representative for the nearest relatives of birds, the evolutionary analysis including outgroup comparison shows, that the PPC of Ratites and Tinamous is primitive within birds. Other groups of birds that are considered primitive within the Neognathae, the Galliformes and Anseriformes (Sibley & Ahlquist, 1990; Sibley & Monroe 1990), are not found close to the Palaeognathae but close to the Ciconiiformes. This was also found by Mindell (1992) after re-analysing the data of Sibley & Ahlquist (1990). From an analysis of the tree at ordinal level it is clear that most members of an order group together. The few that do not, have relatively long evolutionary branches and may be misplaced due to ‘long branch attraction’ (Hendy & Penny, 1989). The ordinal clustering might be the reason why other groups are apparently misplaced. Within the Ciconiiformes the branches are separated by relatively small internodes, while the distances to other orders are relatively large. This difference in branch length might have disturbed the analysis. Although the phylogenetic structure is not clear from this analysis it can be concluded from the ordinal clustering that for each order a prototype PPC can be determined which diverged within the order, resulting in a variety of forms.

Modifications of the PPC are probably highly dependent on its function. This raises the question about the special functional demands that might have resulted in the palaeognathous PPC. The function of the PPC has been described by Bock (1964) for a prokinetic neognathous bird. He showed that the PPC plays a role in the movement of the upper bill. The functional explanations given for the palaeognathous PPC so far are always in the context of rhynchokinesis, a special form of cranial kinesis. In rhynchokinesis only a short, rostral part of the upper bill moves relative to the rest of the upper bill. The rest of the upper bill remains stable relative to the cranium. The discriminating characters found in this analysis confirm the hypothesis of Bock (1963) that the overall palaeognathous PPC configuration is more rigid and strongly built than in Neognathae. This rigid configuration is apparent from the broad Pterygoid-Palate articulation, a broad vomer and broad pterygoids. A strong PPC would be necessary to carry the forces to bend the rather stiff upper bill. Two characters however oppose the hypothesis that the configuration of the palaeognathous PPC is related to rhynchokinesis: the large *Processus basipterygoideus* and the small orbital processes of the quadrate.

Bock (1964) states that the large *Processus basipterygoideus* is a holdover from reptilian ancestors. He suggests that the *Processus basipterygoideus* played an important role in meta- and mesokinesis without explaining what this role might be. Similarly, Bock does not appoint a specific function to the basipterygoid process in birds. Hofer (1945) and Elzanowski (1977) suggested that this process plays an important role in shock-absorption in pecking birds. The process limits the caudal movement of the quadrate and pterygoids, and redirects forces from the quadrate to the cranium. The rostral movement is limited by the *Processus zygomaticus* of the *Os Squamosum* and the relatively diagonal orientation of the merged *Capitula oticum* on the *Processus oticus* of the quadrate. These limitations are at odds with the hypothesis that Palaeognathae are rhynchokinetic; rhynchokinesis implies a freely movable quadrate. The short orbital process of the quadrate also argues against the existence of rhynchokinesis in palaeognathous birds. The *Musculus protractor pterygoidei et quadrati* inserts on the orbital process. This muscle rotates the quadrate forward, resulting in elevation of the upper bill. As the orbital process is rather small, the working arm of the forces generated is small, resulting in relatively small forces to elevate the upper bill. If the forces necessary to bend the upper bill are indeed large (cf. Bock, 1963) it seems unlikely that the Palaeognathae have a highly kinetic bill. Only a kinematic analysis of feeding behaviour can show whether the Palaeognathae do possess a kinetic skull. Furthermore, an analysis of movements and forces working on the PPC and upper bill is necessary to establish a functional relationship between the palaeognathous PPC and rhynchokinesis.

An alternative interpretation for the rigidity of the PPC is that after the loss of two lateral bony bars (postorbital and nasal bar), the palaeognathous skull was reinforced by a much more rigid Pterygoid-Palatinum Complex. During the evolution of birds several elements have slenderised and fenestration has occurred. This process is described by Zweers *et al.* (1997) and includes, starting from the ancestral diapsid/saurian skull, the following phases: 1. a pre-orbital fenestra evolved rostral to the orbit and caudal to the naris. This fenestra is bordered by the lacrimal and jugal bar. 2. The orbit is enlarged, the lacrimal and postorbital bars are slenderised and the anterior fenestra is enlarged. 3. The third or pre-bird stage is characterised by fused postorbital fenestra, bordered caudally by a slender quadrato-jugalquadrato-squamosal bar and rostrally by a slender jugal postorbital bar, large orbits, and slenderised lacrimal bars. In this pre-bird stage four lateral bars are present: the quadrato-jugalquadrato-squamosal bar, the jugal -postorbital bar, the lacrimal bar and the nasal bar. According to Zweers *et al.* (1997) the slenderising of these bars together with the detachment of the secondary palate resulted in the kinetic skull typical for birds. In Neognathae the quadrato-jugalquadrato-squamosal bar has vanished completely and the postorbital bar has disappeared or is replaced by a ligament. In Palaeognathous birds the reduction of lateral elements is even more severe. All bars, except the lacrimal, are either absent or replaced by ligaments. Interestingly, the data available suggest that this continued reduction of bony elements in Palaeognathae is not related to cranial kinesis. However, a broad and rigid PPC would make sense if it is assumed that Palaeognathae secondarily lost their need for cranial kinesis. To make the skull akinetic it is necessary to stabilise the upper bill, through a reinforcement of the skull. The PPC offers one of the few possibilities to reinforce the skull in the absence of a postorbital and nasal bar. Therefore the PPC is considered to reinforce the skull so that movement in the upper bill as a result of external forces due to feeding are limited.

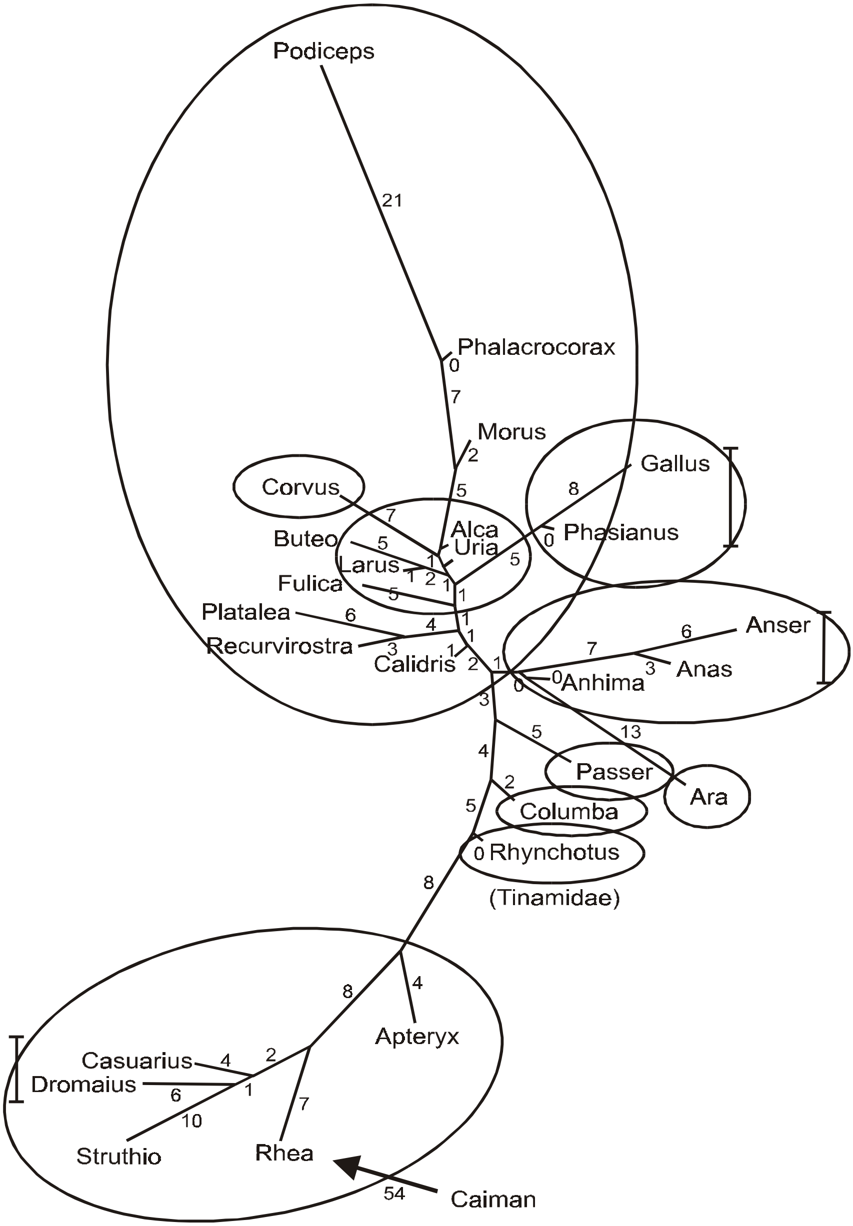

Figure 2.3. Unrooted maximum likelihood tree. The point of attachment of the outgroup is indicated by the arrow and is near Rhea. Numbers indicate the distances between nodes, for clarity nodes with distance nil are indicated with small lines. Systematic units, orders and families, based on molecular data are indicated.

CHAPTER 3

FUNCTIONAL ANALYSIS OF THE RHYNCHOKINETIC JAW APPARATUS IN THE RED KNOT (*CALIDRIS CANUTUS*)

Summary

The Pterygoid-Palatinum Complex (PPC) plays an important role in the elevation of the upper bill. However, it has never been investigated whether the type of cranial kinesis is related to a specific morphology of the PPC. Such a relationship has been suggested for the Palaeognathae, which possess a broad, rigid PPC and a special type of kinesis (central rhynchokinesis). In this paper an analysis is made of the feeding behaviour, cranial kinesis and morphology of the PPC of a distal rhynchokinetic bird. Analysis of the feeding behaviour showed that rhynchokinesis occurs throughout the feeding cycle. The feeding cycle itself resembles strongly the general neognathous feeding behaviour. Remarkable is that cranial kinesis occurs also during swallowing when no food-particles are near the bill tip. This may be the result of a combined action of both the opener and closer muscles of the lower bill. It is concluded that the type of cranial kinesis mainly determines the configuration of the bill, while the morphology of the PPC is mainly affected by the feeding behaviour.

In close collaboration with: M.A.A. van der Meij.

Introduction

A special feature of birds and some reptiles is their ability to move their upper jaw (bill). This upper bill movement, also known as cranial kinesis, is mediated by a complex system that is thoroughly described by Bock (1964). The elevation of the upper bill starts with a forward rotation of the quadrate, which is situated just behind and below the eye. The movement of the quadrate is transferred to the upper bill by two different systems. The first system consists of the jugal bars and transfers the movement onto the premaxilla. The second system, the Pterygoid-Palate Complex (PPC, Gussekloo & Zweers, 1999) is probably the most important and consists of the pterygoids, palate and vomer. The muscles that induce the movement of the upper bill all attach to the PPC or the quadrate.

Differences between types of cranial kinesis are determined by the position of the kinetic zone around which the upper bill rotates. Zusi (1984) describes three types of kinesis that occur in birds. In the most common form, prokinesis, the entire upper bill rotates around a hinge in the nasal-frontal area. The second most common type of cranial kinesis is known as rhynchokinesis. In this type the flexible zone is situated more rostrally and located within the upper bill; the nasal-frontal hinge is no longer kinetic. The third type is known as amphikinesis and can be considered a combination of prokinesis and rhynchokinesis with two flexible zones: one in the nasal-frontal area and a second one just caudal to the rostrum maxillare in the bill itself.

To make rotation in the upper bill possible a number of adaptations is found in the bill of rhynchokinetic birds. To allow rotation around a flexible zone within the upper bill, the movement of the dorsal and ventral bars of the upper bill has to be uncoupled. This uncoupling is achieved by a special arrangement of the bones in the caudal part of the nostril (the lateral bar, see Zusi, 1984). In prokinetic birds the lateral bar is fused with the dorsal bar rostral to the nasal-frontal hinge. This results in an upper bill that can only move as a single unit. In most rhynchokinetic birds the dorsal connection of the lateral bar is shifted caudally, to the area behind the nasal-frontal hinge. Furthermore, a flexible zone is present in the lateral bar and it no longer rigidly connects the dorsal bar with the ventral bar. This makes it possible to slide the ventral bar forward or backward, while the dorsal bar remains stationary. The rhynchokinetic configuration of the lateral bar is known as the schizorhinal nostril, while the prokinetic configuration is described as holorhinal (Garod, 1873).

Zusi (1984) recognised a number of different types of rhynchokinesis based on the number and position of the flexible zone(s) within the upper bill: 1) Double rhynchokinesis, with two flexible zones, 2) Distal rhynchokinesis, with a flexible zone in the distal part of the bill, 3) Proximal rhynchokinesis, with a flexible zone near the proximal end of the bill, 4) Extensive rhynchokinesis, with a large flexible zone in the centre of the upper bill and 5) Central rhynchokinesis. Central rhynchokinesis is only found in palaeognathous birds and is characterised by a narrow flexible zone in the central area of the bill (Zusi, 1984). However, a recent behavioural analysis of one of the Palaeognathae, the Rhea (*Rhea americana*), showed that the flexible zone in the upper bill of this species is large and of the extensive rhynchokinetic type (Chapter 7). Because palaeognathous birds have a very distinct PPC (McDowell, 1948; Bock, 1963; Gussekloo & Zweers, 1999) and a special type of kinesis (Zusi, 1984) several attempts have been made to connect the two (Bock, 1963; Hofer 1954; Simonetta, 1960). However, a functional morphological analysis of rhynchokinetic feeding behaviour has never been performed and a general hypothesis about the relation between the PPC morphology and the type of kinesis is not available.

This study focuses on the relation between the morphology of the PPC and a rhynchokinetic bill as was suggested for the Palaeognathae. To find the relation between PPC morphology and

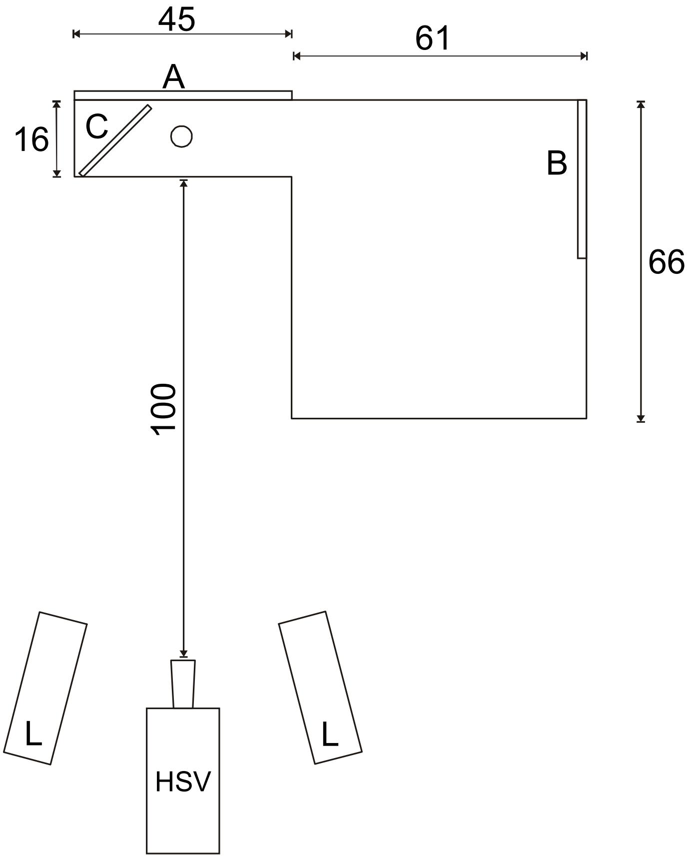

Figure 3.1. Experimental Set-up in top-view. Food-items were offered near the circle. HSV=Highspeed video, L-Cold Light Source, A=reference grid behind the feeding arena, B=reference grid behind the animal, visible in the mirror view, C=mirror at an angle of 45 degrees. All distances are given in centimetres.

a rhynchokinetic bill, an analysis was made of the osteology, myology and arthrology of the PPC of a distal rhynchokinetic bird, the Red Knot (*Calidris canutus*). We tried to identify characters of the PPC that are specific for a distal rhynchokinetic mechanism. To distinguish between features that resulted from rhynchokinesis and those that resulted from the specific feeding behaviour of the Red Knot, the feeding behaviour was analysed and specific functional demands of probing were determined.

Materials and Methods

As model for distal rhynchokinetic birds the Red Knot (*Calidris canutus*) was chosen. The natural history of the Red Knot is well known (Piersma, 1994) and part of its morphology has been described (Burton, 1974; Gerritsen, 1988). The analysis of the morphology of the jaw apparatus was based on dissection of a large number of specimens. Five osteological specimens were used to describe the skull and its elements, fifteen complete heads were used to study the myology, arthrology and osteology. Some of the dissected heads were preserved in 4% formaldehyde, while others had been freshly stored at –20 °C. All preparations were made under a dissection microscope (magnification up to 40 times). To make it easier to distinguish between muscles and other tissues 'Weigert's variation of Lugon solution' was used (Bock and Shear, 1972). This solution colours muscle tissue brown-red, while the colour of ligaments, aponeuroses, veins and nerves remains unchanged. It also simplifies the determination of fibre direction in thin muscle layers. For the nomenclature the second edition of the Nomina Anatomica Avium (Baumel *et al.*, 1993) was used.

The feeding behaviour of the Red Knot was described, and special attention was paid to rhynchokinesis. This behavioural analysis was used to describe specific functional demands related to feeding behaviour (probing) and to rhynchokinesis. For the

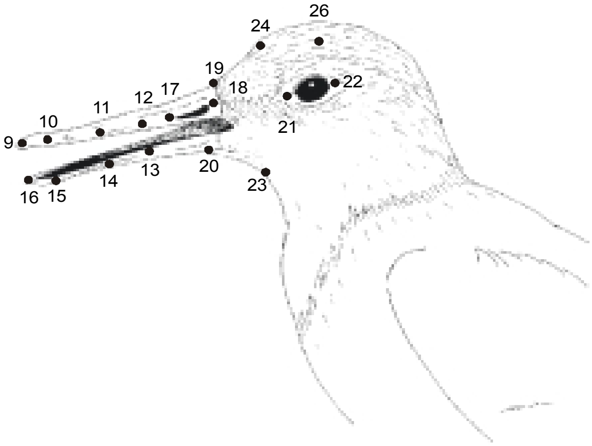

Figure 3.2. Position of the markers placed on the head of the Red Knot (Calidris canutus). Numbers refer to the numbers in table 3.1 All markers were placed on the left side only, except marker 25, which was placed on the right side opposite to marker 26 (visible in mirror view).

analysis of the feeding and drinking behaviour two specimens were used. These birds were kept in the laboratory and trained to feed in the experimental set-up (Fig. 3.1). The feeding of the birds was recorded with a high-speed video camera (500 fps). In each video-frame two images of the bird were visible: one direct image from lateral and one dorsal view obtained from a mirror, which was situated in front of the feeding area at an angle of 45 degrees. For the analysis of the feeding movements a number of white paint markers were placed on the head and bills of the birds (Fig. 3.2, Table 3.1). The position of these markers and the position of the food-item were digitised for every 1/100th of a second of the video-recordings. A reference grid positioned behind the bird, was used to digitise reference points. To assure that a wide range of feeding movements was analysed, food-items of different sizes were offered. The food-types consisted of pellets (Trouvit) to which the birds were accustomed before the experiment. The pellets were cylindrical and of three different sizes: Small, length 5 mm, diameter 0.9 mm; Medium, length 5 mm, diameter 3.5 mm; Large, length 5.0, diameter 5.0. The pellets were covered with a thin layer of chalk to facilitate digitising. Five cycles of each food-type were analysed for each individual.

The digitised points were used to calculate several parameters (Table 3.2), which describe the general movement patterns of the feeding behaviour of the Red Knot. Because of noise in the digitised points rhynchokinesis could not always be determined accurately. To verify our findings about the moments in the feeding cycle when rhynchokinesis occurred, a strain gauge was put on the upper bill of one of the birds and the feeding behaviour was again analysed. During the experiment only medium-sized food-items were offered, and only the most essential markers were digitised so that gape could be determined. The signal of the strain gauge was (pre-) amplified (10x) and digitally stored (5 kHz). The position of the strain gauge was determined after manipulating osteological specimens.

Results

1. Morphological Analysis

General descriptions of the morphology of Sandpipers have been given by Burton (1974), Gerritsen (1988) and Zweers & Gerritsen (1997). In our study we checked these descriptions for the Red Knot and collected additional information on this species where necessary. No extensive morphological description is presented here but a number of features that are related to rhynchokinesis or probing are selected and a qualitative interpretation of their functional significance is given.

1) The bill was long and slender (Fig. 3.3), which makes it possible to penetrate deep into the substrate while total reaction force of the penetration is low (Gerritsen, 1988; Zweers & Gerritsen, 1997). The laterally compressed bill in combination with the rhynchokinetic opening mechanism results also in a reduction of dorso-ventral, external forces during opening of the bill (Gerritsen, 1988). Several features of the bill are designed to resist rostro-caudal reaction forces generated during probing behaviour. Both the dorsal and ventral bar of the upper bill are compressed dorso-ventrally, which results in a lateral stabilisation of the upper bill. Dorso-

ventral stabilisation of the upper bill is achieved by a rostrally elongated inter-nasal septum. This elongated septum stabilises the caudal part of the upper bill so that bending can only occur in the distal part of the upper bill.

2) The quadrate is orientated almost rostro-caudad, in line with the lower bill. This results in a good transfer of caudally directed reaction forces from the pterygoids and palate onto the cranium. The orientation of *Pila otica* in the quadrato-squamosal articulation (Fig. 3.4) ensures that the *Processus oticus quadrati* is in line with the probing direction.

3) The rostral part of the pterygoid articulates with the *Processus basipterygoideus* (Fig. 3.3), which transfers caudally directed reaction forces directly onto the cranium, thereby

Table 3.1. Data obtained from each frame of digitised High-Speed Video. Reference points are given in figure 3.2.

No.	Group	Description
1	Calculated	Earth bound frame of reference, point 0,0.
2		Earth bound frame of reference, point 0,10 (definition y-axis).
3		Earth bound frame of reference, point 10,0 (definition x-axis).
4	Reference grid	Reference frame, point 0,0.
5		Reference frame, point 0,2 (definition y-axis).
6		Reference frame, point 2,0 (definition x-axis).
7	Holding	Food hold point, digitised on grid.
8	Food-item	Position food-item.
9	Upper Bill	Most distal upper bill marker.
10		Upper bill marker.
11		Upper bill marker.
12		Most proximal upper bill marker.
13	Lower Bill	Most proximal lower bill marker.
14		Lower bill marker.
15		Lower bill marker.
16		Most distal lower bill marker.
17	Reference Bills	Rostral border of the nostril.
18		Caudal border of the nostril.
19		Caudal border of the visible part of the upper bill.
20		Caudal border of the visible part of the lower bill.
21	Reference Cranium	Rostral border of the eye.
22		Caudal border of the eye.
23		Throat perpendicular under the eye.
24		Reference triangle, rostral.
25		Reference triangle, dorsal left.
26		Reference triangle, dorsal right.
27	Mirror view	Reference triangle, rostral: in mirror.
28		Reference triangle, dorsal left: in mirror.
29		Reference triangle, dorsal right: in mirror.
30		Upper bill most distal marker: in mirror.

reducing the forces on the pterygoid-quadrate and quadrate-cranium articulations.

4) The palate shows specific features, which can also be explained as adaptations to large caudally orientated impact forces generated during probing. Between the medial surface of the *Arcus jugale* and the connection of the *Processus maxillopalatinus* and the palate a *Pila maxillopalatina* (Fig. 3.5; Johnson, 1984) is present. The presence of this strut was first described by Lowe (1931) and later reanalysed by Zusi and Jehl (1970). They distinguish four different struts on the maxillopalatina (A, B, C, D) of which only strut C is present in the Red Knot (Fig. 3.5). The function of this rostro-caudad orientated strut is to transport caudally directed reaction forces more effectively onto the cranium.

5) The *Processus orbitalis quadrati* is well developed with a large attachment area for the *Musculus protractor pterygoidei et quadrati* (Fig. 3.3), which rotates the quadrate forward and elevates the upper bill. The *Processus orbitalis quadrati* is also relatively long compared to prokinetic birds, which results in a better moment-arm for several muscles attaching to the *Processus orbitalis quadrati*. On the mandibulae an elongated slightly ventrally orientated *Processus retroarticularis* is present (Fig. 3.3) which improves the moment-arm of the *Musculus depressor mandibulae*. This improved moment-arm of the depressor muscle might result in a larger opening force on both upper and lower bill (see Bock, 1964).

Table 3.2. Parameters obtained from the data-points given in table 3.1. These parameters were calculated for each frame of digitised High-Speed Video.

Measurement	**Abbr.**	**Description**
X-pos	X	Horizontal position of the head relative to the earth bound frame (EBF).
Y-pos	Y	Vertical position of the head relative to the earth bound frame (EBF).
Food distance	FD	Distance between the food item and the head.
Gape 1	Gp1	Distance between the upper and lower bill at markers 9 and 16.
Gape 2	Gp2	Distance between the upper and lower bill at markers 10 and 15.
Gape 3	Gp3	Distance between the upper and lower bill at markers 11 and 14.
Gape 4	Gp4	Distance between the upper and lower bill at markers 12 and 13.
Gape 5	Gp5	Distance between the upper and lower bill at markers 19 and 20.
Rhynchokinesis	Rhyn	Angle between distal tip (markers 9 and 10) and proximal part (markers 10 and11) of the upper bill.
Prokinesis	Pro	Angle between proximal part of the upper bill (markers 17 and 18) and the cranium (markers 24 and26).
Kinesis Mandibulae	KM	Angle between distal tip (markers 13 and 14) and proximal part (markers 14 and 15) of the lower bill.

6) The most prominent arthrological adaptation is the presence of bending zones (*Zona flexoria*) in the upper bill (Fig. 3.6; see also Gerritsen, 1988). In the upper bill a *Zona flexoria rostrodorsalis* and a *Zona flexoria rostroventralis* are present. The *Zona flexoria rostrodorsalis* was situated in the dorsal bar of the upper bill, the *Zona flexoria rostroventralis* in both ventral bars of the upper bill. To allow movement of the upper bill, a flexible zone was present in the lateral bars also (*Zona flexoria craniofacialis lateralis*). This flexible zone uncouples movement of dorsal and ventral bars. The bending in the lateral bar is made possible by a caudal shift of

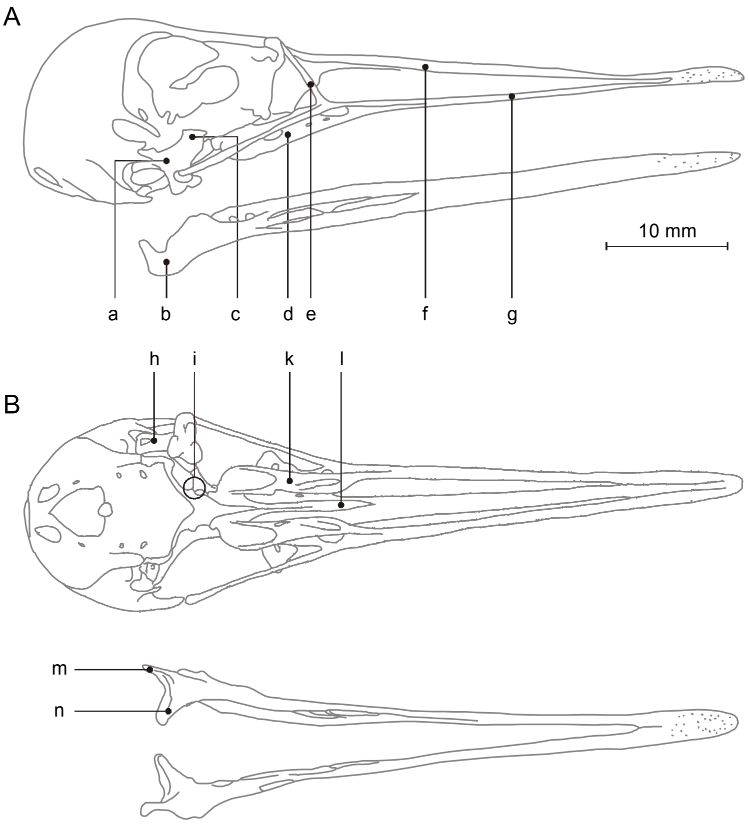

Figure 3.3. Skull of Calidris canutus in lateral (A) and ventral (B) view. The lower bill is seperated from the cranium. Quadrate and pterygoid are removed on the left side in ventral view. a) quadratum, b) Processus retroarticularis, c) Processus orbitalis quadrati, d) palatinum, e) lateral bar, f) dorsal bar, g) ventral bar, h) quadrate, I) Processus basipterygoideus articulating with the pterygoid, k) palatinum, l) vomer, m) Processus retroarticularis, n) Processus medialis.

the base of the lateral bars to a position behind the nasal-frontal hinge as found in schizorhinal skulls (Fig. 3.6; see also Zusi, 1984).

7) Two different flexible zones in the *Rami mandibulae* made mandibular kinesis possible (Fig. 3.6). These zones are the *Zona flexoria intermandibularis rostralis* and the *Zona flexoria intermandibularis caudalis*. The first is situated near the *Rostrum mandibulae*, the second near the *Fenestra rostralis mandibulae* in the most mediolateral flattened part of the ramus. The flexible zones made it possible to depress the tip of the lower bill.

8) Depression of the tip of the lower bill (mandibular kinesis) is achieved by the action of the *Musculus pterygoideus ventralis medialis*, which attached to the distal part of the *Processus medialis mandibulae* (Fig. 3.3). When the muscle contracts, the distal part of the medial process is pulled forward, which results in a outward bending of the central part of the *Rami mandibulae*. This outward bending results in a depression of the distal part of the lower bill. Other parts of the *Musculus pterygoideus* have a different line of action and operate mainly as closer-muscles of the upper jaw. This mechanism has earlier been described for other species such as the herring gull and the barn owl (Bühler, 1981).

9) Large forces for opening the upper bill are achieved by a relatively large *Musculus protractor pterygoidei et quadrati*. This muscle attached to the distal part of the *Processus orbitalis quadrati* thereby improving the moment arm and increasing the opening force on the upper bill.

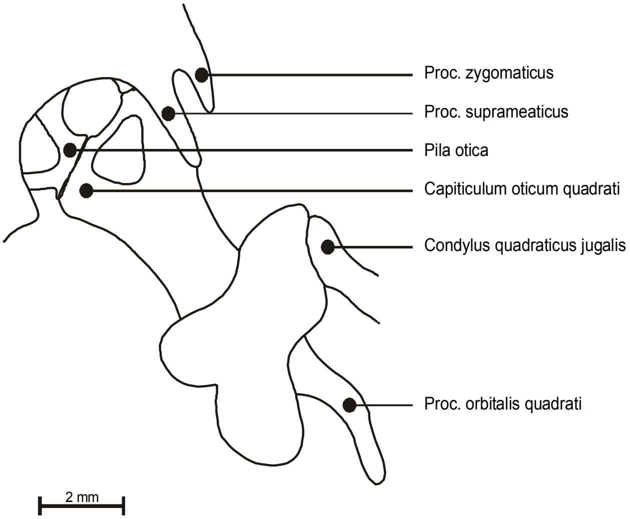

Figure 3.4 Detail of the right quadrato-squamosal articulation in ventral view. Position and orientation of Pila otica in the skull of Calidris canutus

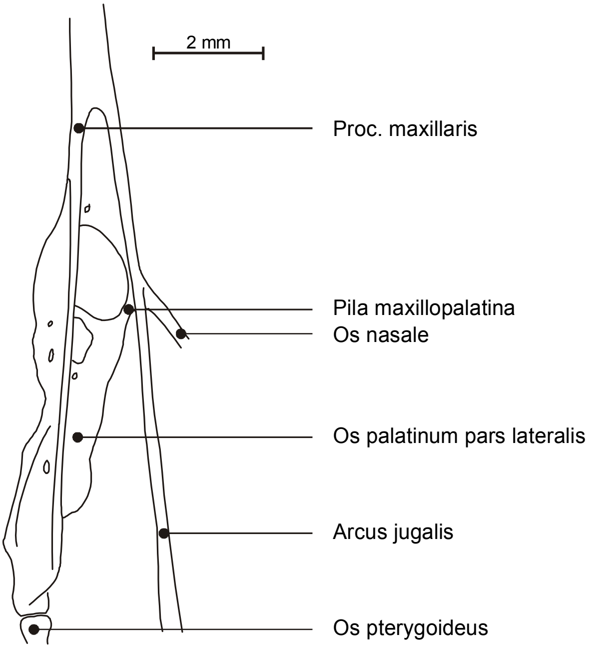

Figure 3.5. Latero-ventral view of the right palate of the Red Knot. Position and orientation of Pila maxillopalatina in Calidris canutus.

10) Even a detailed inspection of the morphology of the pterygoid/palatine/vomer complex did not show any characteristics that can be related to rhynchokinesis as such. The PPC is very similar to that of prokinetic birds, e.g. chicken (van den Heuvel, 1992), pigeon (van Gennip, 1986), or duck (Zweers, 1974). This was also found in a morphometric study of the PPC complex of a large number of birds (Gussekloo and Zweers, 1999).

2. Kinematic Analysis

2.1 General feeding behaviour

The feeding behaviour of the Red Knot follows the general avian feeding (pecking) pattern as described by Zweers *et al.* (1994). Some elements of the feeding behaviour of Sandpipers have been described by Gerritsen (1988).

Feeding behaviour of the Red Knot starts with the approach phase. Several centimetres above the food-item the head is fixated (fixation phase) for a short period. In the subsequent final head approach the bills are opened and orientated in the direction of the food-item while the head approaches the food-item. During the jaw-opening phase both the upper and lower bill show kinesis (Fig. 3.11). When the bills are around the food-item, the bills are closed and the food-item is grasped. Sometimes the bird cannot get a good grip and the food-item is dropped. When the food-item is grasped between the bills correctly, the stationing phase follows: in this phase the food-item is repositioned for intra-oral transport. The repositioning is achieved by a series of 'Catch & Throw' movements (Zweers *et al.*, 1994). During these 'Catch & Throw' movements the food-item is not transported rostrally or caudally. After the food-item is repositioned it is transported through the mouth. Two different mechanisms are used for this transport. The first is a series of 'Catch & Throw' movements, during which the food-item moves more caudally in every 'Catch & Throw' cycle. The second method is a 'Slide & Glue' mechanism (= lingual wet adhesion, Hildebrand *et al.*, 1985). When the jaws are opened the tongue is pushed forward against the food-item (Fig. 3.7). The food-item sticks to the bird's tongue with mucus and is transported backward by a retraction of the tongue. After retraction the tongue has to be repositioned under the food-item for the next retraction. Depending on the size of the food-item the food is transported through the mouth by one of these mechanisms or by a combination of the two. When small food-items are eaten, the food-item is transported with several 'Slide & Glue' movement cycles only. Between 'Slide & Glue' cycles the Red Knot often stops tongue movement to reposition the food-item. Large food-items are transported with a combination of 'Catch & Throw' and 'Slide & Glue'. One or more 'Catch & Throw' movements

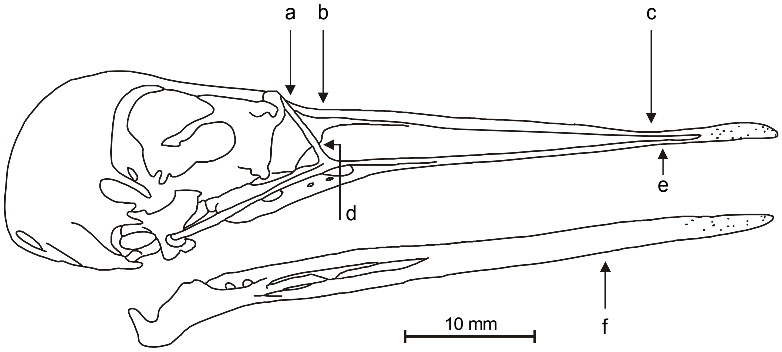

Figure 3.6. Flexible zones in the skull of Calidris canutus in lateral view. The lower bill is seperated from the cranium. a) Zona flexoria craniofacialis lateralis, b) Zona flexoria craniofacialis medialis, c) Zona flexoria rostrodorsalis, d) Zona flexoria nasalis dorsalis, e) Zona flexoria rostroventralis, f) Zona flexoria mandibularis.

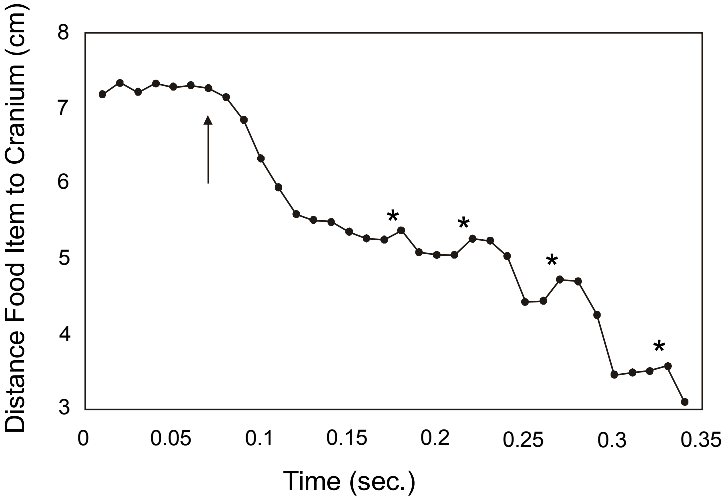

Figure 3.7. Intra-oral transport of a food item. The arrow indicates the grasp of the food item. Asteriks indicate rostral movement of the food item caused by lingual protraction.

are used to transport the food-item to a position near the rictus. Intermediate sized food-items show intermediate behaviour, with in some cases only ‘Slide & Glue’ behaviour and in other cases a combination of ‘Catch & Throw’ and ‘Slide & Glue’. After the intra-oral transport the food-item is transported to the area behind the tongue and by a final retraction of the tongue and larynx into the oesophagus (swallowing).

2.2 Quantitative differences between food-types

The results from both birds are combined after a comparison between the handling times for each food-type. The handling times, defined as the time between grasping the food-item until swallowing, are not significantly different between the two birds. There are, however, significant differences between the handling times of the different food-items (Two-way ANOVA; individual: df=1, F=0.99, p=N.S.; food-types: df=2, F=14.48, $p<0.001$; interaction: df=2, F=1.05, p=N.S.). The average handling time for small food-items is 0.25 ± 0.13 sec., for medium sized food-items 0.54 ± 0.29 sec. and for large food-items 0.77 ± 0.19 seconds. These differences are clearly demonstrated when the position of the food-item relative to the cranium is plotted against time (Fig. 3.8). Figure 3.8 shows that the position of larger food-items remains constant for a long period of time. This constant position is due to repositioning behaviour while the food-item is situated near the bill tips. The actual transport time without repositioning of the food-items is comparable between food-types. Differences in maximum gape are also observed between the different food-types (Fig. 3.9). It is clear that during the transport phase the gape is adjusted for

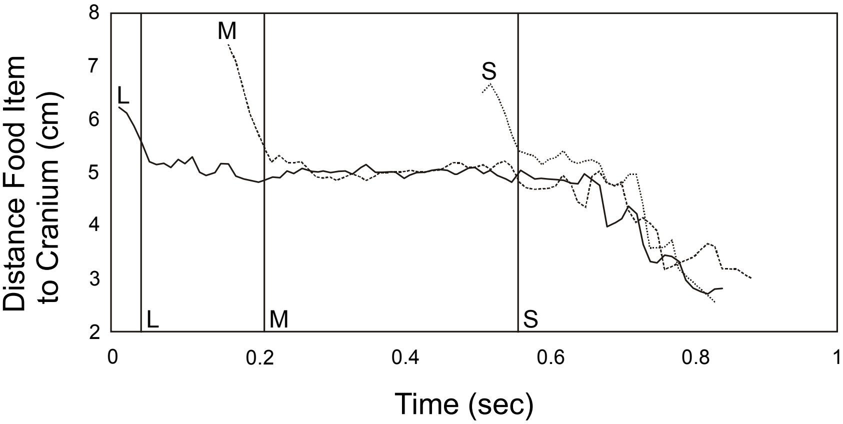

Figure 3.8. Average handling time for different food types. L=Large food items, M=Medium sized food items, S=Small food items. Ventral lines indicate moment of first food grasp.

food size. During transport of the food-item through the bill, the gape increases as the distance to the rictus level decreases.

2.3 Rhynchokinesis

Kinesis in the upper bill of the Red Knot is clearly distal rhynchokinesis. The part of the upper bill that can move relative to the cranium is the small, most rostral part of the bill (approximately 10 mm long; Gerritsen, 1988). The rhynchokinesis in the upper bill is observed mainly in feeding behaviour. During drinking only very small movements in the upper bill were observed. Measurement error in combination with the small changes of the angles in the upper bill made it impossible to quantify rhynchokinesis accurately. It was possible, however, to interpret rhynchokinesis qualitatively. In general there seemed to be a correlation between the size of the gape and the amplitude of rhynchokinesis. This is confirmed by the experiments with the strain gauge, which showed larger strain (=bending) in the upper bill during large gapes (Fig. 3.10). The largest amplitude of rhynchokinesis is observed when the bill is maximally opened.

The moments of kinesis will only be described for small and large food-items (Fig. 3.11). Moments of kinesis for intermediate sized food-items were not consistent. During grasping of large items the rostral part of the upper bill is elevated, which results in an enlargement of the gape. During repositioning the moveable part of the upper bill is depressed (ventral rhynchokinesis) to fix the food-item between the bills and elevated (dorsal rhynchokinesis) to release the food-item. The gapes produced during the transport phase show dorsal rhynchokinesis. These elevations of the bill tip are always smaller than those in the swallowing phase. During swallowing the food-item is located in the very back of the beak, and

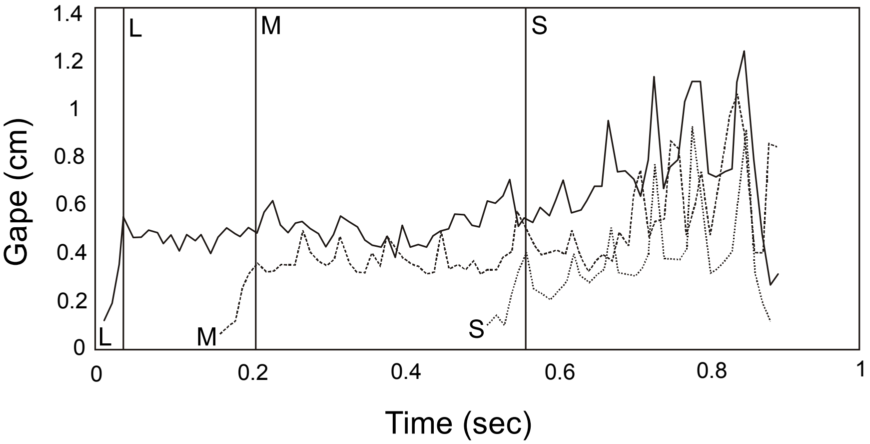

Figure 3.9. Different gapes for different food types. L=Large food item, M=Medium food item, S=Small food item. Vertical lines indicate first food handling.

elevation of the tip of the bill seems to have no direct function. For small food-items only low amplitude rhynchokinesis is observed in both the positioning phase and the swallowing phase.

Mandibular kinesis is always ventrally orientated and can be observed in several stages of the feeding behaviour. When feeding on small food-items mandibular kinesis is only observed during the transport phase. This feature is however much more prominent in the feeding on larger food-items. During feeding on large food-items rhynchokinesis is observed in the repositioning phase, the transport phase and the swallowing phase. Lower bill kinesis is also larger in amplitude during large amplitude gaping.

Discussion

The Red Knot breeds on the arctic tundra and feeds on insects and other non-burrowed prey during that period. Outside the breeding season the birds migrate south to estuary mud flat areas where their main food source becomes burrowed bivalves. During the breeding season the Red Knot is therefore a peck feeder while it becomes a probing bird during winter. The birds possess a kind of cranial kinesis that has been described as distal rhynchokinesis. Distal rhynchokinesis is considered an adaptation to probing behaviour since only a small part of the upper bill has to be elevated in the mud, which reduces the total reaction force on the moving part of the upper bill (Gerritsen, 1988).

The morphology of the jaw-apparatus of the Red Knot has been described by several authors of which the work of Burton (1974) is the most complete. In his work some adaptations for probing behaviour are described but most of them are unclear. Especially the description of the adaptations of the *Musculus depressor mandibulae* is confusing. Burton states that due to the orientation of the medial fibres of the *Musculus depressor mandibulae*, this muscle would

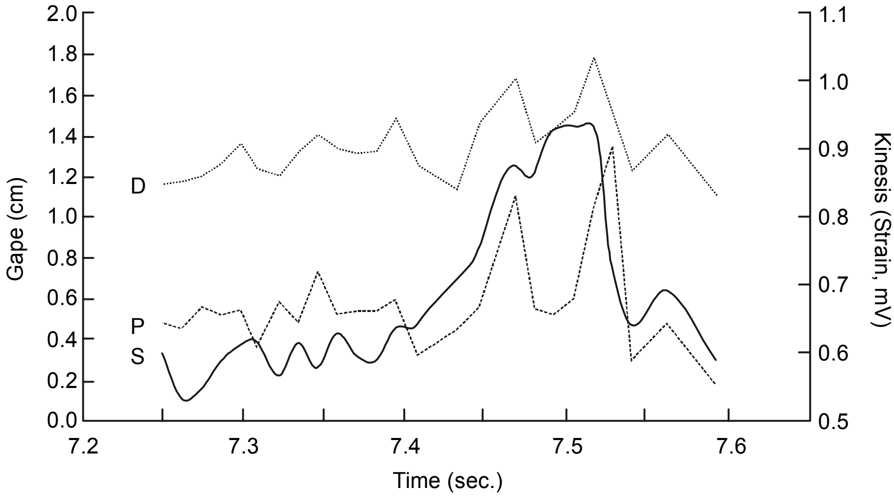

Figure 3.10. Rhynchokinesis during feeding on medium-sized food items by Calidris canutus. Rhynchokinesis is represented by the output of a strain gauge (S) on the flexible zone of the upper bill. Gape is measured from markers on a proximal (P) and a distal (D) point of the upper bill.

have a forward component. Our analysis showed that the origo of the fibres of the depressor muscle, however, was situated caudal to the *Processus retroarticularis* and the *Musculus depressor mandibulae* did not have a forward component. The nomenclature of the *Musculus pterygoideus* of Burton is different compared to ours. Burton divides the *Musculus pterygoideus* into three parts only and combines the *pars dorsalis rostralis* and *pars ventralis lateralis* into a single part called M. The two parts do have a combined insertion on the mandibulae, but the difference in origo and work-line, justifies a distinction of the two parts as separate elements in the muscle complex.

Our morphological analysis showed several adaptations to feeding behaviour within the jaw-apparatus of the Red Knot. However, a distinction must be made between adaptations specific for rhynchokinesis and adaptations to probing behaviour itself. Adaptations for probing behaviour can be divided into two groups. The first group, which facilitates penetration, is mainly found in the bill. The second group, which includes adaptations to resist large forces along the bills and enlargement of muscles that are important in opening the bills, is mainly found in the more caudal part of the jaw apparatus. A previous study of the feeding behaviour of the Red Knot (Gerritsen, 1988) already showed that the bill shape is very important for efficient probing, and that rhynchokinesis is an efficient method for catching and holding burrowed prey (Gerritsen, 1988; Zweers & Gerritsen, 1997). The actual adaptations to make rhynchokinesis possible were however never pointed out. We found that adaptations are mainly found in the bill

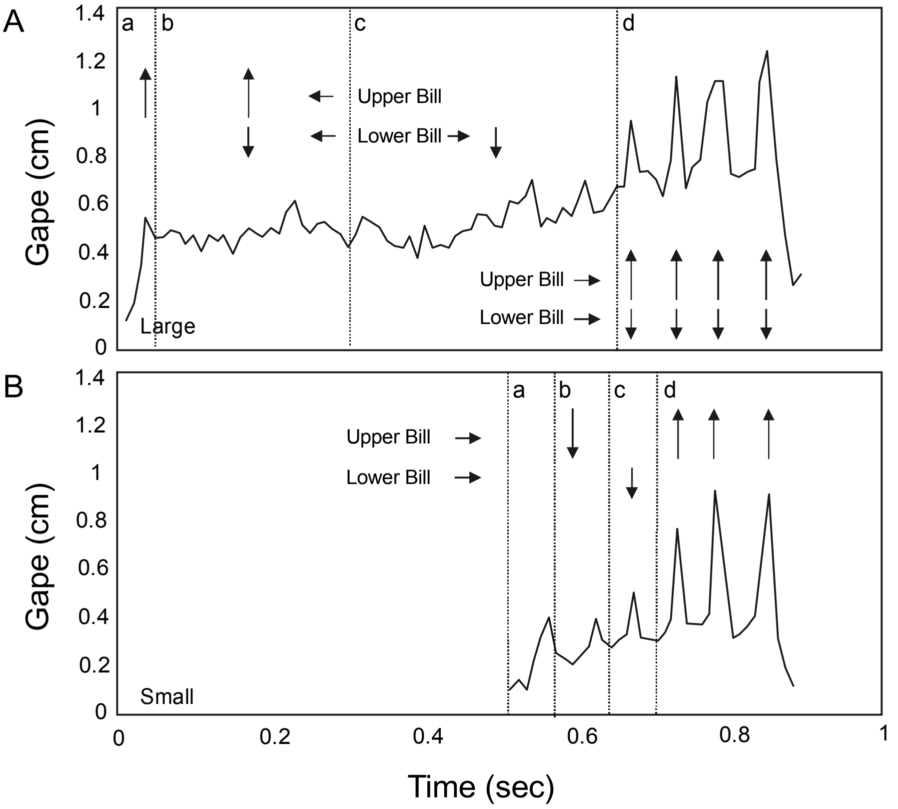

Figure 3.11. Moments of rhynchokinesis during feeding on large (A) en small (B) food items. Feeding cycles are divided in a) grasp, b) repositioning, c) transport, d) swallowing. For phases b and c only presence of kinesis during these phases is indicated (no time value). Upward arrows indicate elevation, downward arrows indicate depression of the upper or lower bill tip.

itself and no clear adaptation to rhynchokinesis could be identified in the Pterygoid-Palate Complex. The adaptations for rhynchokinesis are limited to the schizorhinal nostril, bending zones in the lateral bars and bending zones in both the dorsal and ventral bars. These adaptations have been described earlier (Zusi, 1984) and were clearly present in the Red Knot.

To find the relation between PPC morphology and rhynchokinesis, it is necessary to know the functional demands for rhynchokinesis. The different types of rhynchokinesis (distal, central, proximal, extensive and double) are associated with a large variation in feeding behaviours, and even within a single type of rhynchokinesis feeding behaviour may vary. Proximal and double rhynchokinesis are for example found in both fishing birds (Larinae) and peck-feeding birds (Columbidae; Zusi, 1984). This makes it very difficult to determine a single set of functional demands for rhynchokinesis. Only distal rhynchokinesis is correlated with a specific type of feeding behaviour: all distal rhynchokinetic birds are probe-feeders (see Zusi, 1984). This specific relationship makes it possible to determine functional demands for the upper bill. The lack of a relationship between a specific feeding behaviour and other types of kinesis is somewhat surprising. From a mechanical point of view there is no reason to assume that relatively small differences in the position of the bending zones are associated with principally different functional demands, that is principally different mechanical loading regimes. Reports on rhynchokinesis are often based on manual manipulation of osteological specimen. It is not only very difficult to make an accurate estimate of the position of the bending zone from these manipulations, but especially in lightweight constructions as the avian skull, it is also very difficult to control the force applied. Effectively, any structure will bend if the force applied is large enough. The position of bending zones is hardly ever determined quantitatively and very little, if anything, is known about jaw opening forces that can be produced by different species of birds or the external forces that act on the bills during feeding.

This study showed that the Red Knot has a distal rhynchokinetic skull and that this type of cranial kinesis does occur during feeding behaviour. Rhynchokinesis occurs clearly when the bill is opened during the grasp phase, which can be explained as a functional adaptation to probing feeding behaviour. However rhynchokinesis also occurs in phases in which a functional explanation is not apparent. The most prominent elevation of the upper bill is observed during large swallowing gapes. During swallowing the food-item is situated in the caudal part of the oropharynx, and an elevation of the distal part of the upper bill seems to have no direct functional meaning for swallowing. Furthermore, elevation of the distal upper bill seems to increase with gape amplitude (pellet size). This suggests that in the Red Knot the elevation of the bill tip may be the consequence of a single jaw opening motor pattern In pigeon and chicken the grasp of a food-item starts with quadrate protractor activity followed by depressor activity (Bout & Zeigler, 1994; van den Heuvel, 1992). This sequence of activities is reflected in the kinematics: upper bill elevation precedes lower bill depression. During intra-oral transport and swallowing upper beak elevation is smaller than during grasping or even completely absent and upper beak elevation lags lower bill depression (van den Heuvel, 1992). As pointed out by van Gennip & Berkhoudt (1992) simultaneous activation of adductor (or passive force from stretching) and depressor muscles of the lower bill may also push the quadrate upward and forward. Although EMG's of the Red Knot are not available, co-activation of adductor and depressor muscles during jaw opening has been described in the pigeon. Especially in the Red Knot, the force generated by muscles other than the quadrate and pterygoid protractors may generate extra force to open the upper beak during probing. The rostro-caudal orientation of the quadrate in probers is very suited for the transfer of force to the quadrate and it is speculated here that adductor-depressor co-activation is a standard part of the beak opening motor pattern in the Red Knot. The effect of adductor/depressor co-activation is illustrated by a simple static model of the quadrate and mandible. The configuration (orientation and distances) of muscles and bony elements for the rest situation was measured (see fig. 3.12). By choosing the depressor force to be 1 N in each position the free body diagram of the entire system contains three unknowns: the x and y component of the reaction force in the quadrate-skull articulation and the adductor force. The sum of the moments of adductor and depressor muscle around the quadrate-skull articulation is zero when the adductor is 0.44/0.40 = 1.1 times the depressor activity (the ratio of the moment arms). For adductor/depressor activity ratios >1.1 the quadrate will be pushed forward and elevate the upper bill. If the lower bill has to depress at the same time, the moment of the depressor around the quadrato-mandibular joint has to be larger than that of the adductor muscle. By the same reasoning, the adductor force must be < 1.3 (0.88/0.68) times the depressor force. For 1.1 $F_{Depr} < F_{Add} < 1.3 F_{Depr}$ both beaks will open. The elastic forces generated by elevation of the upper bill will be transferred through the PPC to the quadrate and potentially counteract the quadrate movement. This force was not included in the model, but is very small over the physiological range of upper bill elevations (Nuijens & Bout, 1998; Chapter 6).

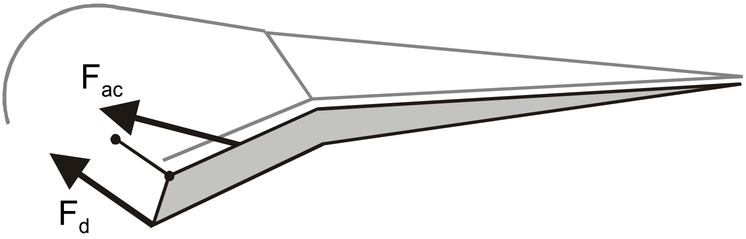

Figure 3.12. Static mathematical model in rest position. F_d=Force of the Musculus depressor mandibulae, F_{ac}=Force of the Musculus adductor mandibulae externus complex.

The evolution of the different types of cranial kinesis in birds is still unclear, but the general opinion has been that prokinesis is the most primitive condition (e.g. Bock, 1964). A recent theory states that the ancestor of all living birds was a ‘transitional shorebird’ (Feduccia, 1995). Based on this hypothesis an evolutionary pathway for the evolution of bills adapted to probing behaviour has been proposed which assumes a prokinetic ancestor for rhynchokinetic birds (Zweers and Gerritsen, 1997). The adaptations described in their evolutionary pathway are mainly based on the presence of rhynchokinesis and adaptation of bill shape. Our analysis showed that these are indeed the adaptations found in the Red Knot, with a number of additional adaptations in the jaw-musculature and the morphology of the bony elements to resist the large reaction forces of the mud. Our present study also showed that adaptations for rhynchokinesis are mainly found in the bill itself. If we look at evolutionary pathways from pro- to rhynchokinesis we can therefore focus on the bill itself. The changes in the bill during the transition from prokinesis to rhynchokinesis have always been described as a gradual rostral shift of the bending zones in the bill. Besides the shift of the bending zone, another adaptation must take place to get a functional rhynchokinetic bill, which is the uncoupling of the movement in the ventral and dorsal bars. Although the shift of the bending zones is probably similar for all the types of rhynchokinesis, the uncoupling of the ventral and dorsal bars has occurred in at least two different ways. While most types of rhynchokinetic skulls possess a schizorhinal nostril, which makes uncoupling of the dorsal en ventral bars possible, in birds with central or palaeognathous rhynchokinesis this uncoupling is achieved by a gap in the lateral bar (Bock, 1963; Zusi, 1984). This indicates that these types of rhynchokinesis are probably not homologous or derived within the same lineage. The non-homology of rhynchokinesis is also confirmed by the presence of rhynchokinesis in a wide range of different systematic groups (see Zusi, 1984). A single evolutionary pathway for the origin of rhynchokinesis cannot be postulated. For the sandpiper-like distal rhynchokinesis it has been shown that certain adaptations are present which improve probing and prey handling. It is however very well possible that other types of rhynchokinesis, or the same rhynchokinesis in different habitats, may have a completely different function. We therefore conclude that the mechanism of development of rhynchokinesis is similar (rostral shift of flexible zones and uncoupling) for all types of rhynchokinesis but that the evolutionary forces responsible for each type may be different. Each potential rhynchokinetic morphology must therefore individually be checked for flexible zones in the upper bill, uncoupling of the ventral and dorsal bar, and specific functional demands. Only when these three features are known a clear evolutionary pathway can be determined.

Acknowledgements

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CHAPTER 4

A SINGLE CAMERA ROENTGEN STEREOPHOTOGRAMMETRY METHOD FOR STATIC DISPLACEMENT ANALYSIS

Summary

*A new method to quantify motion and displacement of bony structures has been developed, since quantification is often difficult due to overlaying tissue, and the currently used roentgen stereophotogrammetry method requires significant investment. In our method, a single stationary roentgen-source is used, as opposed to the usual two, which, in combination with a fixed radiogram cassette holder, forms a camera with constant interior orientation. By rotating the experimental object, it is possible to achieve a sufficient angle between the various viewing directions, enabling photogrammetric calculations. The photogrammetric procedure was performed on digitised radiograms and involved template matching to increase accuracy. Co-ordinates of spherical markers in the head of a bird (*Rhea americana*) were calculated with an accuracy of 0.12 mm. When these co-ordinates were used in a displacement analysis, relocations of about 0.5 mm could be accurately determined.*

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Introduction

A special feature of birds is their ability to move not only their lower beak, but also their upper beak. This in contrast to mammals in which only the lower jaw can move. The upper bill motion in birds is controlled with a series of articulating rigid elements, known as the Pterygoid-Palate Complex (PPC; Gussekloo & Zweers, 1999). The morphology of the Pterygoid-Palate Complex is well known for most birds (see Baumel *et al.*, 1993) and its function in upper bill kinesis has been qualitatively described for neognathous birds (Bock, 1964; Zusi, 1984). However, the actual displacements of bony elements in the PPC during elevation of the upper bill have never been measured. The main reason why this has never been done is because displacements are very small and the essential elements are covered with skin or other overlaying tissues, which can interfere with the analysis of movement. In the past this problem was usually solved by simple radiophotography and radiocinematography (e.g. Heidweiller & Zweers, 1990). The great disadvantage of these methods is that results are always two-dimensional. In medical research, several three-dimensional methods are available, such as Magnetic Resonance Imaging (MRI), Computed Tomography (CT) and roentgen-stereophotogrammetry. Both MRI and CT are simpler in their procedure than roentgen-stereophotogrammetry, but they require expensive equipment and give images with insufficient resolution to determine small displacements. The cost involved in roentgen-stereophotogrammetry analysis are much lower, and since the method results in much higher resolution images, it is the preferred method to detect small changes. To make a three-dimensional reconstruction using roentgen-stereophotogrammetry, radiograms of an object in at least two different directions are necessary. In medical research accurate results have been achieved using this technique (e.g. Axelsson *et al.* 1996; Nillson & Kärrholm, 1996; Hilding *et al.*, 1996; Möll & Garwics, 1995; Kiss *et al*., 1996; Axelsson *et al.*, 1997; Önsten, 1995; Ilchman *et al.*, 1995). The majority of these studies were based on the work of Selvik (1989) and used two roentgen-cameras. A roentgen-camera consists of a roentgen-source in combination with a receiver, usually a radiogram cassette. When only one camera can be used at a time, the two photographs have to be taken with the same camera in sequence. Since the object has to be photographed from different angles, either the camera or the object has to be moved. The normal procedure in both aerial and close-range photogrammetry is to move the camera. In aerial photogrammetry this is the only option, while in close-range photogrammetry the easiest solution is to move the camera, since it is small and the interior orientation (relation between lens and film) of the camera remains constant (Atkinson, 1996). In roentgen-photogrammetry two problems arise when the position of the camera is changed. In a normal single lens camera an image is acquired on the basis of a central projection, which means that at a certain position in the camera all rays entering the camera cross at one point. In normal cameras this point is the optical centre of the lens or focus, and the object is outside the camera. In roentgen-cameras, however, the point of central projection is the radiation source and the object is located between the central projection point and the film. It is clear that, when moving a normal camera, the position of the focus relative to the film does not change, since both are in the same apparatus. In a roentgen-camera,

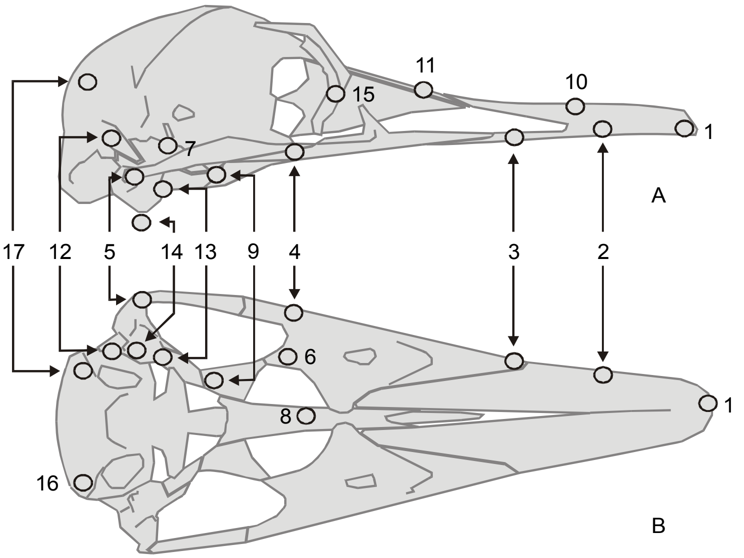

Figure 4.1. Distribution of the markers inside the experimental object. The skull is projected in lateral (A) and ventral (B), markers are only shown when their position is visible in the views shown. Numbers correspond with the numbers in table 4.1. In radiograms all markers are visible, unless their projection coincides with the projection of another marker or a metal rod.

however, these two elements are uncoupled and when source and receiver are not displaced in exactly the same manner, a change in the interior orientation will occur, which makes analysis more complex. To overcome these problems we developed a roentgen-stereophotogrammetry method, which makes it possible to calculate three-dimensional co-ordinates of spherical markers using a single position-fixed roentgen-camera with constant interior orientation.

The purpose of the analysis is to determine relocations of markers between two conditions of the experimental object. When analysing relocations, it is not only necessary to determine three-dimensional positions of markers in two conditions, but also to detect significant changes in these co-ordinates between the two conditions. This was done using displacement analysis, a method normally used in geodetic analysis. In this study, the position of the markers was determined at different moments in time to reconstruct a movement.

To test the procedure, displacements in the skull of a large bird, the Rhea (*Rhea americana*), were determined. The movement chosen for reconstruction was the opening of the upper bill. Bill opening was represented by the start or rest condition (‘Closed Bill’) and the final or elevated condition (‘Elevated Bill’), and displacements were induced by pulling the upper bill open. This roentgen-stereophotogrammetry procedure can be used in any kind of animal or tissue in which markers can be placed and the object fixed and rotated.

Materials and Methods

The new roentgen-stereophotogrammetry procedure described consists of a series of methods. The total procedure determines the three-dimensional relocation of markers between two different situations of the experimental object. In this study, the experimental object was the skull of a large bird, the Rhea (*Rhea americana*), and the two positions were the skull with the upper bill in rest position and the skull with the upper bill in elevated position. Positions of markers were calculated using a roentgen-stereophotogrammetry method based on six radiograms. Co-ordinates were calculated using a bundle-adjustment analysis. The differences between conditions were calculated using a displacement analysis. All elements of the procedure are described below.

Table 4.1. Position of the markers

No	Bone	Position
1	*Os premaxillare*	Rostral
2	*Os premaxillare*	Ventral bar near the *Rostrum maxillare*
3	*Os premaxillare*	Centre of the ventral bar
4	*Os jugale*	Rostral
5	*Os quadratojugale*	Caudal
6	*Os palatinum*	Rostral
7	*Os pterygoideus*	Caudal
8	*Vomer*	Central
9	*Os palatinum*	Caudal
10	*Os premaxillare*	Dorsal bar near the *Rostrum maxillare*
11	*Os premaxillare*	Centre of the dorsal bar
12	*Os quadratum*	*Processus oticus*
13	*Os quadratum*	*Processus orbitalis*
14	*Ramus mandibulae*	Caudal
15	*Os ectethmoid*	Central
16	*Os parietale*	
17	*Os parietale*	

The head of a deceased Rhea (*Rhea americana*) was chosen as experimental object for this study. The large number of elements in the PPC of birds necessitates a large number of markers to trace the motion of each element. An advantage of the relatively large size of the Rhea is that markers are not clustered closely together, thus facilitating the identification of individual markers. To attain maximum contrast in the radiograms between markers in the skull and surrounding tissues, the eyeballs and related muscles and skin were removed to facilitate roentgen-penetration. All other aspects of the skull were kept intact during the experiment.

To measure the position of the bony elements accurately, small markers were placed inside each element. The markers were small cobalt spheres with a diameter of 0.8 mm (1/32-inch). For each marker, a small incision in the overlaying tissue was made and a small hole was drilled into the bone. The marker was glued into the hole using alpha-cyanoacrylate adhesive. Incisions in overlaying tissues were restored using small amounts of the same adhesive. For this study 17 markers were placed (Fig.4.1, Table 4.1). Two markers were situated bilaterally on the caudal part of the cranium and were considered stable in all co-ordinates independent of the position of the upper bill. A third marker in the *Os ectethmoidale* was considered as a semi-stable marker since it is not part of the moving system but may show small dislocations due to deformations in the facial part of the skull. Other markers were placed in bony elements that are considered important in the elevation of the upper bill (Bock, 1964; Zusi, 1984). To facilitate the identification of individual markers in the radiograms, markers were positioned only on the right-hand side of the skull.

The roentgen-source (Siemens Gigantos) had focus dimensions 0.6 x 0.6 mm, and a radiation opening-angle of 20°. For image capturing Kodak X-OMAT MA films were used, in combination with a Kodak MIN-R2 cassette. The experimental set-up (fig. 4.2) resembles the set-up as first described by Selvik (1989), combined with the additions made by Huiskes *et al.* (1985) and Ateshian *et al.* (1991). However, the method described here was completely redeveloped using regular geodetic photogrammetrical procedures. Two reference frames were used for the photogrammetric analysis: one calibration frame to determine the interior orientation of the camera, and an object frame to determine the exterior orientation of the object with respect to the camera.

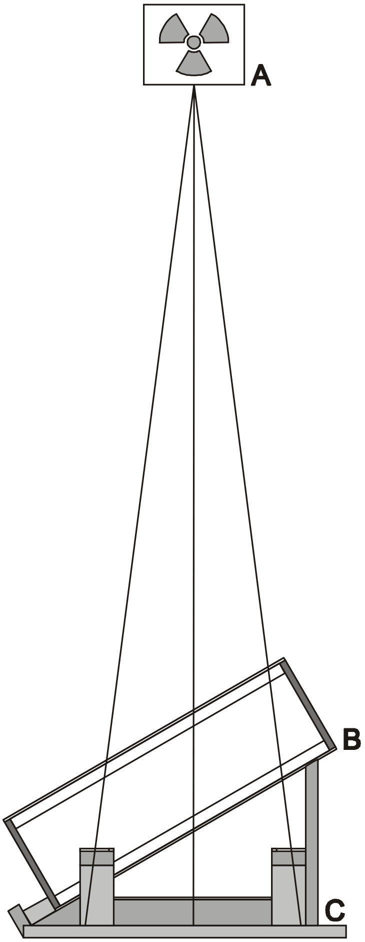

Figure 4.2. Experimental Set-up. The roentgen source (A) is positioned above the calibration frame (C) at a height of 2.15 m. Depending on the radiograph, the object frame (B) is placed on the calibration frame at an angle of –30°, 0° or 30° relative to the horizon.

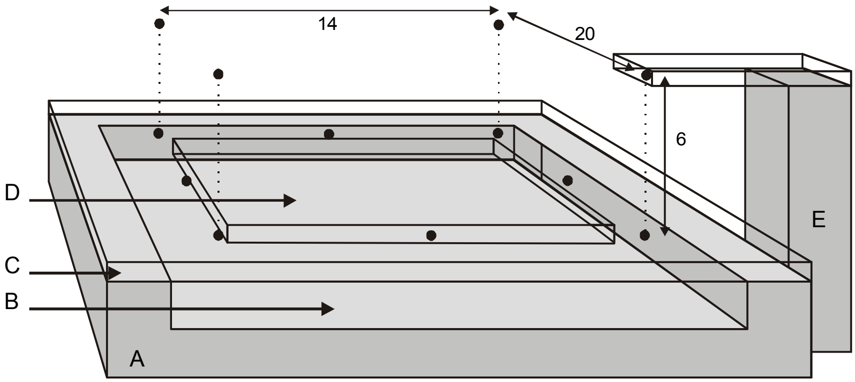

Figure 4.3. The calibration frame. The frame has a base (A) that holds the cassette (B). On top of the frame a PMMA plate (C) is mounted with the centre part missing (D). On the sides four pillars (only one drawn: E) support the upper markers. Black dots indicate the twelve markers in the calibration frame. All measures are in centimetres.

The calibration frame (Fig. 4.3) had a double function. It was used to fix the cassette at a standard distance to the focus, and at a relatively constant position within the camera, and it was used to determine the interior orientation of the camera. Directly above the radiogram cassette a PMMA (Polymethylmetacrylate) plate was present, in which eight markers were placed in a rectangular pattern of 20 x 14 cm. A marker was placed at each corner and at the centre of each side. To reduce the number of layers the roentgen-radiation had to penetrate, a major part of the centre (19 X 13 cm) of the PMMA plate had been removed. Exactly above the four corner markers, six centimetres higher, four other makers were situated in smaller PMMA plates. Two support structures were attached to the calibration frame to place the object frame above the calibration frame at an angle of 30^{O} (Fig. 4.2). The calibration frame was fixed to the ground; the roentgen-source was centred above the calibration frame at a height of 215 cm. This combination of roentgen-source and calibration frame including film is here after referred to as ‘the camera’.

The object frame (Fig. 4.4) was 40 cm long, 12 cm wide and had a height of 13 cm. It consisted of two parallel PMMA plates, thickness 3 mm, situated above each other. On each short side these plates were connected by thick acrylate plates to strengthen the frame and to carry the fixation system for the objects. Along the long sides at each edge and perpendicular to the PMMA plates, a small PMMA bar was made to increase the stiffness of the frame.

Six markers were placed in each PMMA plate. In both plates markers were placed at the centre of the long sides, one centimetre inwards and at the centre of the short side 10-cm inwards. Two additional markers were placed 3 cm next to the central marker on the short side. The two additional markers in each plate were placed diagonally on the other side of the centre

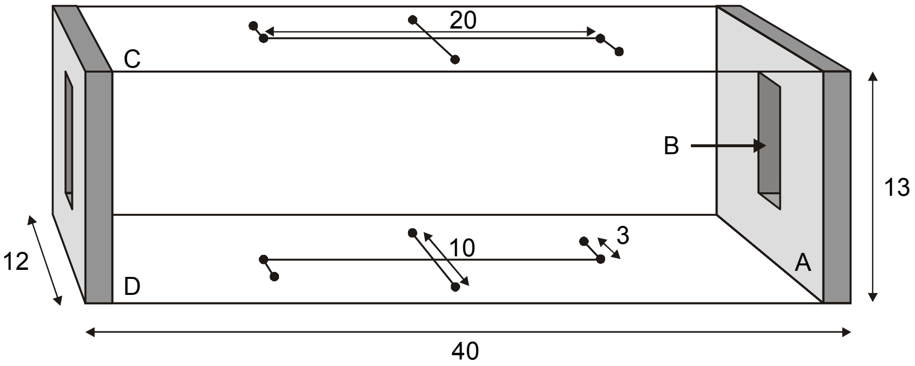

Figure 4.4. The object frame. The frame consists of two support sides (A) in which a space (B) is made for the attachment of the experimental object. The frame further consists of a top PMMA plate (C) and a bottom plate (D). In these two plates a pattern of markers was made as shown in the figure. All measures are in centimetres.

marker. The markers in the lower plate were placed in such a way that the diagonal markers of the upper plate were not in the same position.

The experimental object was connected to the object frame by means of a clamp attached to a metal rod that was attached to one of the acrylate side panels. The clamp could be tightened using a screw. Both the vertical position and the length of the rod could be adjusted. The other side of the object could be fixed to another metal rod, which allowed the same position adjustments as the clamp. To secure the experimental object in the object frame, the clamp was partly inserted into the *Foramen magnum* of the skull and was tightened around the basioccipital bone. This resulted in a stable fixation of the skull. To improve the grip, the *Condylus occipitalis* was removed. Surgical tape was used to fix the rostral part of the skull to the metal rod.

Although two two-dimensional projections from different viewing directions are sufficient to calculate the marker co-ordinates, accuracy can be increased using a larger number of projections, creating redundancy. To obtain this redundancy, radiograms were taken in six different directions through the object in each condition. The orientation of the object in the six radiograms was as follows (all elevations of the object are at an angle of 30^{O}): 1. Dorso-ventral, rostrally elevated; 2. Dorso-ventral, caudally elevated; 3. Dorso-ventral, no elevation; 4. Lateral, rostrally elevated; 5. Lateral, caudally elevated; 6. Lateral no elevation.

Figure 4.5a shows the relative orientation for two of these radiograms, as if the object was stationary and the camera position had changed. This resulted in an angle of 60^{O} between viewing directions. In the actual experimental set-up the camera was stationary and the object was rotated $+30^{O}$ and -30^{O} relative to the horizontal plane, which gave the same result as changing the camera position 60^{O}. Roentgen-source settings were 65 kV and 10 mAs. To

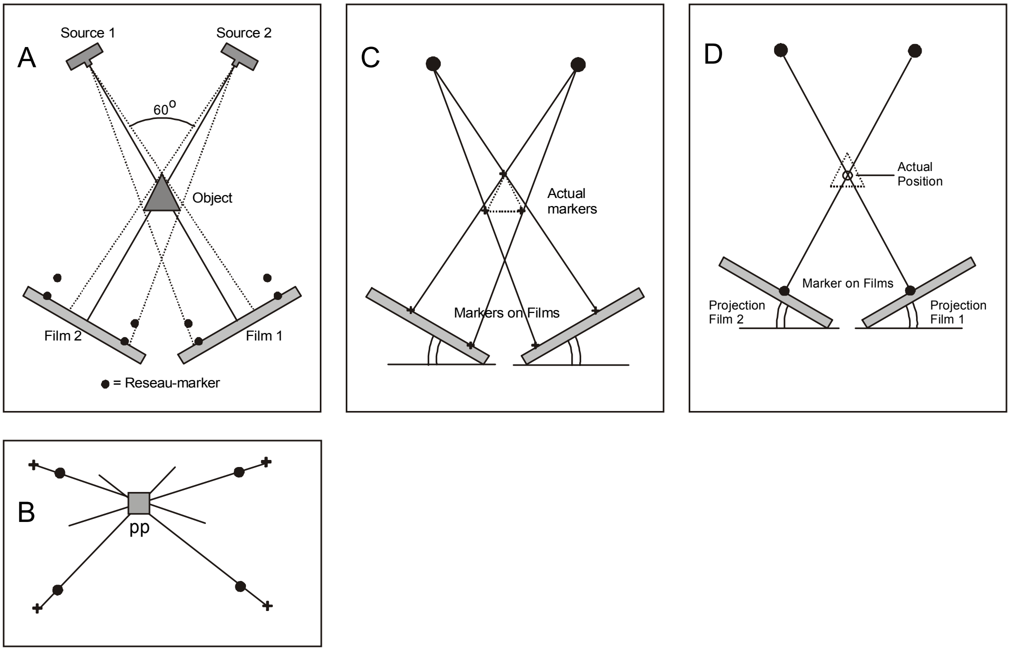

Figure 4.5. Schematic representation of the steps in the analysis. A. (side view of experimental set-up). Six radiograms were taken at an angle described in the text. For simplicity only the radiograms with angles of $+30^{O}$ *and* -30^{O} *are shown. B. (top view on radiogram). Using the projection of the calibration frame markers the principle point (PP) was estimated and using the distance between upper and lower calibration frame markers the height of the source was calculated. C. (side view of experimental set-up). Using known position of the source, the known orientation of the markers in the object frame and the projection of these markers, the actual position of the object frame relative to the camera (exterior orientation) was calculated. D. (side view). Knowing the orientation of the object frame relative to the camera and the position of the camera it was possible tot calculate the lines through the projection of the object marker and the source for all images. The point where these lines intersect was the reconstructed three-dimensional position of the object marker.*

optimise contrast between tissues and markers, these settings may have to be changed for other objects.

The radiograms were digitised with a Kodak DCS420 digital camera (Gruen *et al.*, 1995). The camera sensor resolution was 1012 x 1524 pixels. Camera pixel dimensions were 9 x 9 micrometer, and the geometry of the pixels in the resulting image was known. Since a radiogram of 15 x 24 cm is captured on 1012 x 1524 pixels, a marker with a diameter of approximately 0.8 mm had an area of about 20 pixels. After measurement of the pixel co-ordinates of the points of the calibration frame in the images, the image was resampled to a resolution of 640 x 960 pixels, to correct for rotation (non-parallellity) between the radiogram and the digital camera sensor. For this rectification, a projective transformation from a plane to another plane, the markers on the calibration frame were used. The markers of the calibration frame were known both in the digitised radiogram (measured) as well as in reality (fixed). The images were transformed to a plane in the calibration frame and can be considered to have been taken in this plane.

To calculate the three-dimensional co-ordinates of the markers, the two-dimensional co-ordinates of the markers in the radiograms had to be measured. These co-ordinates of markers in radiograms were preferably measured by means of template matching in digitised images. As described by Østgaard *et al.* (1997), the use of computer-assisted digitising improves the accuracy. In this method an image of the projection of a marker is used as a template, which is used in an optimising algorithm to locate very accurately the position of markers in the digitised radiogram. Similar techniques have recently been described by Vrooman *et al.* (1998). This method, however, requires a good contrast between surrounding tissues and the actual marker. When the contrast between background and markers was poor, the markers were measured manually (Measurement errors; Manual 0.1 mm, Template matching 0.003 mm). Marker co-ordinate measurements were done at the sub-faculty of Geodetic Engineering of the Delft University of Technology.

Bundle adjustment (determination of interior and exterior orientation and three-dimensional co-ordinates)

In the bundle adjustment an iterative optimisation of parameters describing the relationships between actual three-dimensional co-ordinates and their two-dimensional projection is performed (Fig. 4.5c). Using the found parameters the co-ordinates of unknown markers can also be calculated (Fig. 4.5d). Similar techniques have also been described by Huiskes *et al.* (1985), Selvik (1989) and Athesian (1991). The relationships between the three-dimensional co-ordinates and their projections on a two-dimensional plane are based on the mathematical laws of the central projection. Provided that the marker, the projection centre and the marker projection are on a single line, the following relationships, also known as collinearity equations, can be deduced:

$$y = f.\frac{r_{12}(X - X^0) + r_{22}(Y - Y^0) + r_{32}(Z - Z^0)}{r_{13}(X - X^0) + r_{23}(Y - Y^0) + r_{33}(Z - Z^0)} + y^0$$

$$x = f.\frac{r_{11}(X - X^0) + r_{21}(Y - Y^0) + r_{31}(Z - Z^0)}{r_{13}(X - X^0) + r_{23}(Y - Y^0) + r_{33}(Z - Z^0)} + x^0$$

with

f	:	focal length
r_{ij}	:	elements of the rotation matrix from the camera to the object system
X^0, Y^0, Z^0	:	co-ordinates of the projection centre in the object co-ordinate system
X, Y, Z	:	co-ordinates of the marker in the object co-ordinate system
x, y, f	:	co-ordinates of the marker projection in the camera co-ordinate system
x^0, y^0	:	co-ordinates of principle point (=orthogonal projection of projection centre [source] onto the image plane) in camera co-ordinate system

Parameters of the interior orientation and the exterior orientation (X^0, Y^0, Z^0, r_{ij}) were iteratively optimised to minimise the sum of squares of residuals. This optimisation needs an initial value for the parameters describing the interior orientation (x^0, y^0, f) and an estimate of the parameter of the exterior orientation. The parameters describing the interior orientation of the camera (x^0, y^0, f) were calculated using the difference of projection of low and high lying markers of the calibration frame (known as ‘fall over’). The lines through the projection of the high and low lying markers of all four corners intersect at the principal point, which is the location of the perpendicular projection of the focus or radiation source centre (x^0, y^0; Fig. 4.5b). Based on the distances between the projections of the high and low markers and the real spatial distance, the angles of projection were calculated, and from these angles the distance between the theoretical projection plane and focus (f). Since the position of the object frame within the camera was known, approximate values of the parameters describing the exterior orientation (X^0, Y^0, Z^0, r_{ij}) could easily be estimated. The estimated values were accurate enough for relative marker positions within the object, and can be used when the exact position of the object in an external reference system is not important. If an external reference is required, the exact position of the object frame relative to the calibration frame must be calculated.

Displacement Analysis (comparison of conditions)

For a displacement analysis first the co-ordinates of the markers in the experimental object have to be determined in the conditions to be compared. After a transformation in which a reference part of the object, or base, in both conditions was aligned, the displacement between conditions could be calculated. The two conditions in our experiment were ‘Closed Bill’, in which the upper bill is in its rest position, and ‘Elevated Bill’ in which the upper bill is elevated by an external constraint at the bill tip.

For the displacement analysis the ‘Delft’-method was used (Baarda, 1968). Within this method the first step was to calculate co-ordinates in each separate condition using bundle-adjustment. Once this step had been performed as described above, the formal displacement analysis began. In this analysis the co-ordinates of the markers in both conditions were compared to determine the differences. The co-ordinates of both conditions were put into the same co-ordinate frame based on a set of markers (or base) with constant relation independent of the position of the bill. First, the stability of the base was tested by transforming the base co-ordinates of the second condition onto the first and determining the error. To do this, at least three markers have to be stable, and these markers must not be collinear or nearly collinear and can best be distributed evenly over the object. A larger number than three is however always preferred. In this analysis it was presumed that three stable markers were placed in the neurocranium and the ectethmoid bone. The analysis showed that the chosen base was not completely stable and that small dislocations of the marker in the ectethmoid bone had occurred. Although the base was not completely stable, it was used anyhow, since in the highly kinetic bird skull very few completely stable points are present. When the parameters of the base transformation were accepted the same parameters were used to transform all other co-ordinates into the same co-ordinate system. Whether a certain marker has been relocated between conditions depends on the euclidean distance the marker has shifted between conditions and the accuracy of this distance. A point is considered stable when the euclidean distance between the marker in each condition, divided by the propagated standard deviation of this distance, is smaller than the critical value of the normal distribution ($p<0.05$).

Table 4.2. Displacement analysis. Change of visible points in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated distance in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

No	dX	dY	dZ	Dist 3D	s.d.	Dist /s.d.	Moved
2	3.01	0.09	10.92	11.32	0.92	12.30	Y
3	2.79	-0.15	2.77	3.94	0.68	5.79	Y
4	2.15	0.30	0.23	2.18	0.52	4.19	Y
5	0.44	0.84	0.35	1.01	0.57	1.77	Y
6	2.17	0.45	-0.05	2.21	0.49	4.51	Y
7	1.56	0.28	0.47	1.66	0.50	3.32	Y
8	-0.25	-0.13	-0.06	0.29	0.53	0.55	N
9	2.30	-0.13	-0.39	2.33	0.49	4.76	Y
10	1.71	0.00	8.78	8.94	0.88	10.16	Y
11	-0.78	-0.28	-0.04	0.83	0.63	1.32	N
12	0.12	0.26	0.13	0.32	0.50	0.64	N
13	2.06	0.02	0.71	2.18	0.50	4.36	Y
14	0.13	0.89	0.81	1.21	0.65	1.86	Y
15	0.47	0.11	-0.15	0.50	0.32	1.56	N
16	0.00	0.00	0.00	0.00	0.39	0.00	N
17	0.00	-0.04	0.00	0.04	0.38	0.10	N

Results

Bundle adjustment and co-ordinates

After the rectification a good fit was found between the actual co-ordinates of the calibration frame and the calculated co-ordinates in all images of both the ‘Closed Bill’ and ‘Elevated Bill’ condition (Global test [one dimensional F test], $\gamma=80\%$, $\sigma_{a\ priori}=0.1$ mm, df=8, $p<0.001$). The transformed images could therefore be used to determine co-ordinates. All the calculated co-ordinates are tested for their accuracy using individual W-tests (one-dimensional test, using normal distribution). Some markers were not clearly detectable in some images. These markers showed a high W-statistic ($W>1.96$, $p>0.05$). These points were not used in any further analysis, because they could not be accurately measured. Consequently, some markers were only known in either the ‘Closed Bill’ or ‘Elevated Bill’ condition and could therefore not be used in the displacement analysis. When this was the case, the markers were ignored in both conditions. Among these markers were the two at the very tip of both upper and lower bill. The projections of the rostral fixation rod sometimes interfered with the projection of the markers at the bill tips. The remaining points all had an acceptable W-value ($W<1.96$, $p<0.05$) and the systems of both conditions (‘Closed Bill’ and Elevated Bill’) in total were accepted using a global test (Global test, $\gamma=80\%$, $\sigma_{a\ priori}=0.1$ mm, df=13, $p<0.001$). It has to be noted that markers measured using template matching received a lower W-value than manually measured points. The mean calculated standard deviation of the measurements per co-ordinate was 0.12 mm. Using the propagation rule the precision of a marker relative to the Stochastical-base was $0.12\sqrt{3}=0.21$ mm.

Displacement analysis / Stability Analysis

In the stability analysis the two markers in the cranium were used as stable markers (marker no. 16 & 17). The presumed stable marker in the ectethmoid bone (marker no. 15) showed some small dislocations that resulted in a worse fit than could be expected from a base of three completely stable markers. The markers used formed an acceptable stable base for comparison (Global test, $\gamma=80\%$, $\sigma_{a\ priori}=0.1$ mm, df=2, $p<0.001$). The mean accuracy of the co-ordinates after transformation was 0.29 mm.

Displacement analysis / Condition comparison analysis

The final step of the displacement analysis consisted of the comparison of the ‘Closed Bill’ and ‘Elevated Bill’ conditions. To do this the co-ordinates of the stable base in the ‘Elevated Bill’ condition were transformed to fit those of the ‘Closed Bill’ condition. The co-ordinates of the markers were then compared for significant change. In table 4.2 the relocations in three dimensions are given for each marker. When the relocation was larger than the critical value of the global test, the point was considered to have moved significantly. When the standard deviation of a marker in one condition was larger than the relocation distance between the conditions, it was not clear whether that marker had moved or not. However, the movement, if present, was limited and considered small enough to be negligible. The accuracy of the

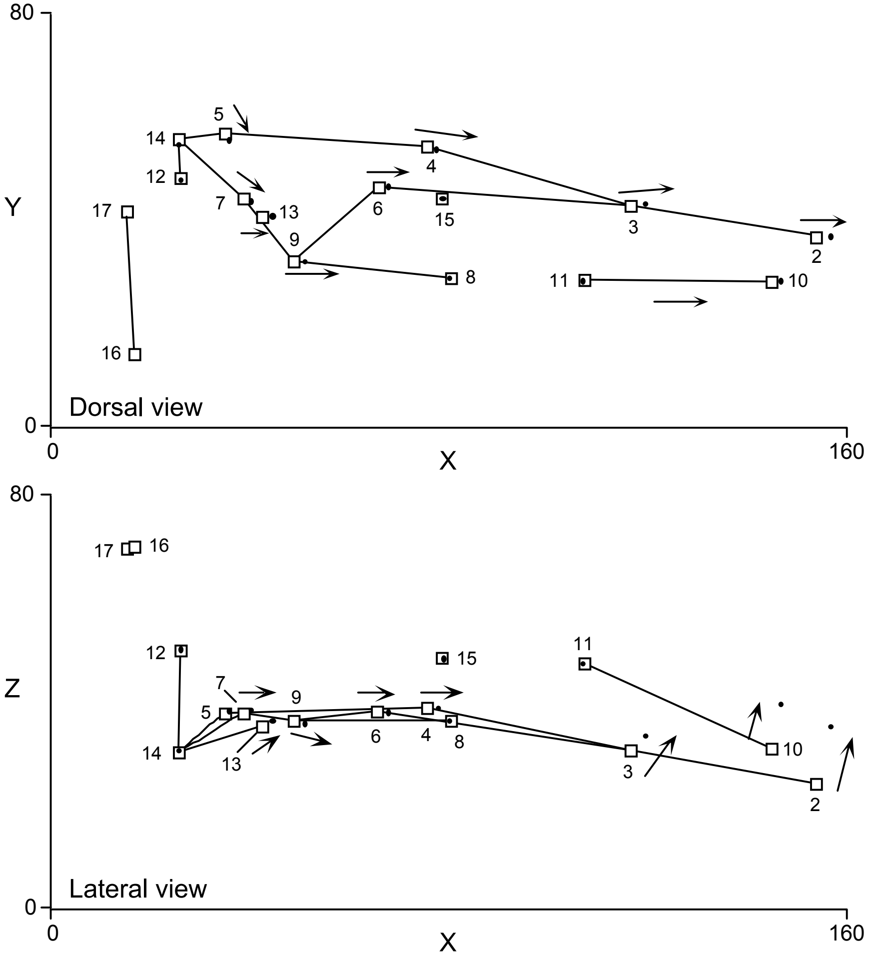

Figure 4.6. Distribution and relocation of the markers in the X-Z plane (Lateral view) and the X-Y plane (Dorso-ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of markers in the 'Elevated Bill' condition. Numbers correspond with the numbers in table 4.1.Drawn lines are schematic indicators of bony elements of the skull. Distances on the axes are given in millimeters.

calculated distances depends on the standard deviations of both markers and was therefore on average 0.56 mm.

In figure 4.6 the positions of the markers in the skull are presented as projections on perpendicular planes. It is clear that large relocations have occurred in the rostral part of the skull, and even the small relocations near the quadrate can be detected (e.g. marker 5). The figure clearly indicates that the Quadrate and Palate-Pterygoid Complex in Rhea moves forward when the upper bill is elevated. A more detailed account of the biological implications of these movements will be described in chapter 5.

Discussion

Our experimental set-up uses a single roentgen-camera thus reducing cost and space, since it obviates the necessity of a second camera. The interior orientation of the camera remains constant, thus allowing the use of standard photogrammetric software. The constant interior orientation also decreases the number of steps in the calculations and thus increases accuracy. For the accuracy of the calculations it is of great importance that the markers in the frames are positioned with the greatest precision and that they are tightly fixed. This can only be achieved with stiff thermostable materials which in most cases are also highly X-ray absorbent. This results in a trade-off between stiffness and visibility. In our case the 3-mm PMMA proved to be sufficient since no deformations were measured in the object frame, but accuracy might be improved by using a more rigid material, which still allows X-ray transmission. Besides determining the exterior orientation, the object frame also protects the experimental object against any unwanted deformations.

The accuracy of the procedure is highly dependent on the camera configuration, the image resolution, the number of markers on the frames, the number of radiograms and the contrast between markers and surrounding tissues. The markers that were measured using template matching had a much higher precision than those measured by hand. Since template matching is highly dependent on contrast between markers and surroundings to make an accurate match, it is necessary to maximise the contrast between them.

To compare two conditions (‘Closed Bill’ and ‘Elevated Bill’) it is necessary to have a stable base to which the two conditions can be calibrated. During the experiment, it was found that the orientation of the head within the frame changed, due to the external forces applied. The object frame could therefore not be used as a stable base. Within the skull, two markers, situated in the caudal part of the cranium, were completely stable. As third point a marker in the ectethmoidal bone was used, which proved to be influenced by bill movement and was therefore not completely stable. Accuracy could be improved by the addition of a third completely stable point at a considerable distance from the two in the cranium. In the Rhea, however, no stable points in the total facial region of the skull are present.

In this experiment the precision of the markers was approximately 0.12 mm. This value is low compared with the accuracy found in other roentgen-stereophotogrammetry research (Axelsson *et al.* 1996; Nillson & Kärrholm, 1996; Möll & Garwics, 1995; Kiss *et al*., 1996; Axelsson *et al.*, 1997; Önsten, 1995; Ilchman *et al.*, 1995) and normal medical close-range dual-camera photogrammetry (Ghosh & Poirier, 1987). This accuracy was achieved by using a larger number of photographs in the bundle adjustment, and applying template matching to increase the accuracy of the measurements in the photographs.

As described by de Lange *et al.* (1990) the accuracy of the measurements, is highly dependent on the number of markers and the configuration of the markers inside one element.

In this experiment, where most elements are bar-like, they are only described by their most rostral and caudal parts. By adding a number of extra markers, especially in the stable cranium, the accuracy of the procedure could be further increased.

This increase in accuracy and in redundancy could be useful in the displacement analysis, since the transformation of a reference part from one situation to another, in which both have a certain deviation, diminishes the accuracy. In this study the accuracy proved to be sufficient to determine relocations of 0.6 mm.

Displacements in the cranium of the Rhea were well detected and described, even when changes were relatively small. Since the accuracy of marker co-ordinate calculations after transformation is smaller than 0.25 mm and the accuracy of the distances between conditions is approximately 0.5 mm the method can be applied to various other studies of discrete displacements or deformations.

Acknowledgements

We like to thank M. Heijmans for carefully making the object and calibration frame, dr. ir. C.I. Spoor for his critical comments on the manuscript and Mrs. D van Bijsterveld and Mrs. A. Fisher for checking the English grammar.

CHAPTER 5

THREE-DIMENSIONAL KINEMATICS OF SKELETAL ELEMENTS IN AVIAN PROKINETIC AND RHYNCHOKINETIC SKULLS DETERMINED BY ROENTGEN STEREOPHOTOGRAMMETRY

Summary

Several different types of cranial kinesis are present within modern birds. These types are characterised by the position of the rotation point. The Pterygoid-Palatinum Complex (PPC) plays an important role in the transfer of movement from the quadrate to the upper bill. The taxon Palaeognathae is characterised by a very distinct PPC morphology, which is very different from that of Neognathae, and a special type of kinesis (central rhynchokinesis). We investigated whether kinematics of the PPC differs in different types of cranial kinesis, which might indicate specific selective forces that explain the morphology of the Palaeognathous PPC. We found that in all investigated types of kinesis (prokinesis, distal rhynchokinesis, central rhynchokinesis) the movement pattern is similar and no differences in functional demands on the PPC, as a result of different types of kinesis, could be determined. In all types of kinesis the PPC moves almost exclusively forward and backward thereby elevating or depressing the upper bill. We therefore conclude that the difference in PPC morphology must be the result of other selective forces than those acting on the movement and that all types of cranial kinesis have probably evolved from a single kinetic ancestor.

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Introduction

Cranial kinesis can be found in a limited number of vertebrate groups such as reptiles and birds. Especially in birds, cranial kinesis is well developed and found in almost all species. In birds cranial kinesis always implies the ability to move the upper bill, or a part thereof, relative to the braincase. Within birds three main types of cranial kinesis have been described (Zusi, 1984): 1) Prokinesis, in which the upper bill itself is inflexible and rotates around the nasal-frontal hinge, 2) Amphikinesis, in which the entire bill rotates around the nasal-frontal hinge with additional rotation near the rostrum maxillae, 3) Rhynchokinesis, in which rotation occurs rostral to the nasal-frontal hinge and a clear hinge is absent. Rotation takes place around flexible zones in the bones of the upper bill.

Within rhynchokinesis a subdivision is made on the basis of the position of the bending zones (Zusi, 1984). Five different types can be distinguished (Zusi, 1984): 1) double rhynchokinesis in which two bending zones are present, one near the nasal-frontal area and a second near the rostrum maxillae, 2) Distal rhynchokinesis in which a single bending zone near the rostrum maxillae is present, 3) Proximal rhynchokinesis in which a single bending zone near the nasal-frontal area is present, 4) Central or Palaeognathous rhynchokinesis in which a single bending zone is present near in the central area of the upper bill, and 5) Extensive rhynchokinesis in which the bending zone extends over the complete area between rostrum maxillare and nasal-frontal area.

A complex mechanism of bony elements, muscles and ligaments generates the movement of the upper bill. This complex includes the quadrates, pterygoids, palates, jugal bars and all associated muscles and ligaments. The closely associated pterygoid and palate will be referred to as the Pterygoid-Palate Complex (PPC; Gussekloo & Zweers, 1999). The role of the quadrate and PPC has been described for a prokinetic bird (Bock, 1964). In this description movement of the quadrate is considered to induce the movement of the upper bill. Rostro-dorsal rotation of this element results in a rostral movement of the pterygoids, which transfer the rostral movement onto the palate, vomer, maxilla and premaxilla resulting in an elevation of the upper bill. Depression of the upper bill could be achieved by a caudo-ventral rotation of the quadrate and a subsequent caudal movement of pterygoid, palate, vomer, premaxilla and maxilla.

Very little is known about the cranial kinesis of the Palaeognathae. This group consists of the Ostrich (*Struthio camelus*), Rheas (*Rhea spec*. and *Pterocnemia spec*.), the Emu (*Dromaius novaehollandiae*), Cassowaries (*Casuarius spec*.), Kiwis (*Apteryx spec*.) and the approximately fifty species in the family Tinamidae (Tinamous; Sibley & Monroe, 1990). According to Zusi (1984) two types of rhynchokinesis can be found only within the Palaeognathae. Tinamous are thought to have extensive rhynchokinesis while all the other Palaeognathae are thought to be central rhynchokinetic. This interpretation was based on osteological specimens of the skulls.

In addition to these special types of rhynchokinesis the species of the Palaeognathae also possess a remarkable PPC, different from that found in neognathous birds (McDowell, 1948; Bock, 1963; Gussekloo & Zweers, 1999). Although several authors have studied the system (Hofer, 1954; Simonetta, 1960; Bock, 1963) no clear function of the special PPC morphology of

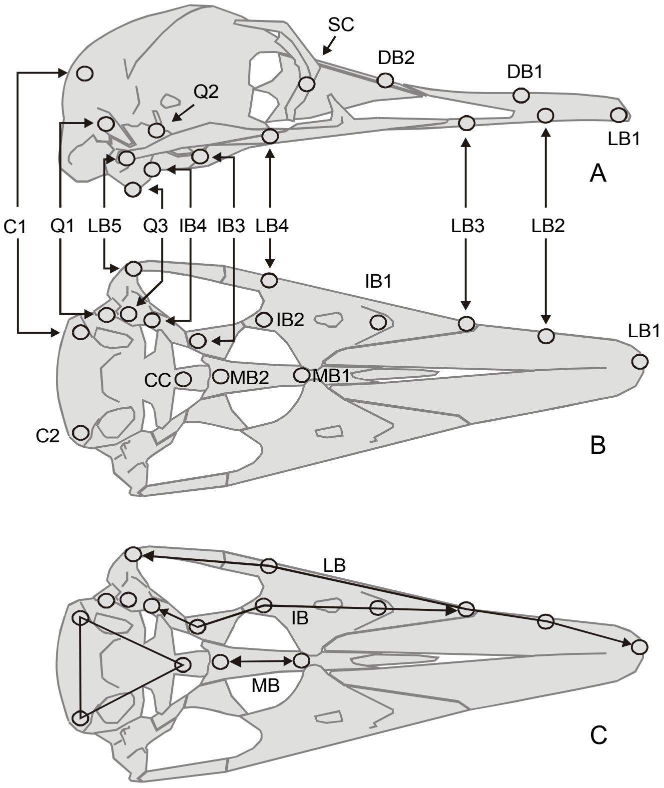


Figure 5.1. Distribution of the markers inside the experimental object. The skull is projected in lateral (A) and ventral view (B), and bars in ventral view (C). Markers are only shown when their position is visible in the views shown. Letter coding correspond with the coding in table 5.1. In radiograms all markers are visible, unless their projection coincides with the projection of another marker or metal rod. LB=Lateral Bar, IB=Internal Bar and MB=Medial Bar.

the Palaeognathae has ever been identified. It is possible that the function of the PPC in Palaeognathae is completely different from that of the prokinetic neognathous birds, for example the reinforcement of the skull (see Gussekloo & Zweers, 1999). The description of the movement of the PPC elements has never been tested experimentally. Differences in types of kinesis or morphology of the PPC maybe associated with differences in the kinematics of these elements. To test whether the PPC serves the same kinematic function independent of the type of kinesis, the displacement of bony elements of the PPC was measured in birds with three different kinds of kinesis. These types are prokinesis, distal rhynchokinesis as one of the most common forms of rhynchokinesis and finally central or palaeognathous kinesis because of its special morphology. Displacement will be measured in three dimensions using a roentgen-stereophotogrammetry technique (Gussekloo *et al.*, 2000).

Table 5.1. Markers placed in the skulls of the experimental specimen. Positions of markers are also given in figure 5.1.

System	Marker	Bony element	Position
Lateral Bar	LB1	*Os premaxillare*	Rostral
	LB2	*Os premaxillare*	¼ bill length from tip
	LB3	*Os premaxillare*	At fusion with Palate
	LB4	Jugal bar	Rostral
	LB5	Jugal bar-quadrate articulation	Lateral
Internal Bar	IB1	*Os premaxillare*	
	IB2	*Os palatinum*	Proc. maxillare
	IB3	*Os palatinum*	Pars lateralis
	IB4	*Os pterygoideum*	
Medial Bar	MB1	*Vomer*	Rostral
	MB2	*Vomer*	Caudal
Dorsal Bar	DB1	Dorsal bar of the upper bill	Rostral
	DB2	Dorsal bar of the upper bill	Caudal
Semi-Constant	SC	*Os ectethmoidale*	Lateral
Medial Constant	CC	*Rostrum paraspenoidale*	Caudal/Proximal
Lateral Constant 1	C1	*Cranium*	Caudo-lateral
Lateral Constant 2	C2	*Cranium*	Caudo-lateral
Quadrate	Q1	*Proc. oticum*	Central
	Q2	*Proc. orbitalis*	Distal
	Q3	*Proc. mandibularis*	Distal

Materials and Methods

The Species

The kinematics of three different types of kinesis was analysed. The most common form, prokinesis, is characterised by rotation of the complete upper bill around a nasal-frontal hinge. In this analysis prokinesis was represented by the Crow (*Corvus corone*). A qualitative description of the kinesis of this species has already been given by Bock (1964). The type of kinesis in which only the rostral part of the bill rotates (distal rhynchokinesis) is mainly found in the order Charadriiformes. This mechanism was analysed in the Red Knot (*Calidris canutus*). The third type of kinesis, found in Palaeognathae only, has conventionally been described as central rhynchokinesis. The kinematics of the PPC was determined in three species of the

Palaeognathae: the Ostrich (*Struthio camelus*), the Emu (*Dromaius novaehollandiae*) and the Rhea (*Rhea americana*).

Of each species a skull of a deceased individual was used. The skin and eyes were removed from all specimens to improve the contrast of roentgen-images. In each specimen 20 cobalt spheres with a diameter of 0.8 mm (1/32 inch) were placed (Fig. 5.1, Table 5.1). The markers (object markers) described 7 sub-systems important in the movement of the upper bill and some served as reference markers. To secure the position of the markers they were attached to bony elements. If necessary, an incision was made in the overlying tissue, and a small hole was drilled into the bone in which the marker was immobilised using alpha-cyanoacrylate adhesive. Large incisions in muscles were closed using a very small amount of the same adhesive. Due to morphological differences it was not possible to place 20 markers in each species, therefore some markers are missing in some species. Some markers were not inserted into the bone completely, and were found inside the overlying tissue after the experiment. These markers were not used in the analysis. An overview of the markers that were used in each species can be found in tables 5.2 to 5.6.

The Procedure

To determine kinematics of the elements in the skull during kinesis three-dimensional co-ordinates of the markers were calculated in two conditions. These conditions represent the rest position of the bill (‘Closed Bill”), and the condition in which the upper bill is elevated (‘Elevated Bill’). Stereophotogrammetry methods were used to determine the exact position of the markers in both conditions, and a displacement analysis was used to quantify the differences between the two conditions.

Stereophotogrammetry is a method in which three-dimensional co-ordinates are calculated from multiple two-dimensional projections. Since in this study the markers are embedded in bone, roentgen-imaging was used. A single roentgen-source was used to obtain images of the experimental object in three different directions. Images were made in sequence and sufficient differences in projection-angle were obtained by rotation of the experimental object while the roentgen-camera remained stationary (1. image from lateral; 2.image from dorsal, rostral end 30 degrees elevated; 3. image from dorsal, caudal end 30 degrees elevated). This method was described in detail by Gussekloo *et al*. (2000), therefore only a brief outline of the procedure is given here.

The complete experimental set-up is shown in figure 4.2. It consisted of a roentgen-source, an object frame and a calibration frame. The roentgen-source in combination with the calibration-frame is called ‘the camera’. Elements of the camera are stationary and do not change position relative to each other or relative to the environment. The object frame (Fig. 4.4) was used to fix the heads during the analysis, and to make elevation of the upper bill possible. The head was attached to the object frame using a small metal rod with a clamp on one end. The clamp was inserted into the *Foramen magnum* and tightened around the basioccipital bone. In some cases the *Condylus occipitalis* had to be removed for proper fixation. Both the length and the vertical position of the rod could be adjusted so that the position of the head could be changed. On the rostral side of the head a second metal rod, of which the length and vertical position could also be changed, was used to fix the bill tips and to elevate the upper bill in the elevated condition. The bill tip was attached to this second metal rod with surgical tape. The specimens were analysed with the upper bill in rest position and with the upper bill elevated. The upper bill was elevated by moving the second metal bar dorsally within the object frame. The amplitude of the elevation was as large as possible without applying excessive force. Some markers were placed inside the object frame to determine the exact position of the frame relative to the camera.

The calibration frame (Fig. 4.3) was used to determine the position of the roentgen-source relative to the film. This was achieved by eight markers situated right above the film and four additional ones at 6 cm above the film. The exact orientation of these markers was known and from the projection of these markers on the film the position of the roentgen-source could be calculated.

The three-dimensional co-ordinates of the object markers are calculated from three two-dimensional projections of the object from different positions. Based on the projection of the calibration-markers it is possible to determine the relative position of the roentgen-source in each radiogram. The projection of the known co-ordinates of the object frame can then be used to determine the orientation of the object-frame relative to the camera. When the orientation of the object frame is known in two directions three-dimensional co-ordinates can be calculated. Since the co-ordinates of the object-frame are known, the solution for the three-dimensional co-ordinates can be optimised. The parameters of the optimised solution are used to calculate the co-ordinates of the other markers (for further detail see Gussekloo *et al.*, 2000). As described above three radiograms were taken of the experimental object. This number is less than used by Gussekloo *et al.* (2000), but still sufficient for accurate determination of co-ordinates and reduced the amount of time necessary for the analysis.

Displacement Analysis (comparison of conditions)

When the co-ordinates of markers are known in both the ‘Closed’ and ’Elevated’ condition the changes in the position of the markers have to be determined. This is done using a displacement analysis. In a displacement analysis the markers of both the ‘Closed’ and ‘Elevated’ condition are transformed in such way that known stable markers (or base) are at the same co-ordinates. After this transformation the change in co-ordinates between conditions can be calculated.

First the base was tested for stability. The base in our experiment consisted of markers in the neurocranium and, when less than three were present, in the ectethmoid bone. The analysis showed that the chosen base was not completely stable and that small dislocations of the marker in the ectethmoid bone had occurred. Although the base was not completely stable, it was used anyhow, since in the highly kinetic bird skull very few completely stable points are present.

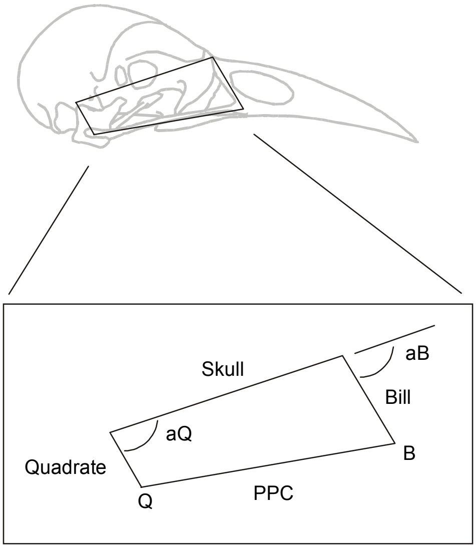


Figure 5.2. Terminology of the four-bar system. The four elements are called 'skull' (connection between the two rotation points in the skull), the quadrate, the PPC and the bill (lateral bar, or connection between the dorsal and ventral Zona flexoria maxillare). Q is the articulation point between the quadrate and the PPC, B is the connection point between the PPC and the 'Bill'-bar. aQ and aB are the angles of the 'Quadrate'-bar and the 'Bill'-bar with the skull.

Whether a marker has been displaced between conditions depends on the Euclidean distance the marker has shifted between conditions and the accuracy of this distance. A point is considered stable when the Euclidean distance between the marker in each condition, divided by the propagated standard deviation of this distance, is smaller than the critical value of the normal distribution ($p<0.05$).

Differences in co-ordinates between the 'Closed bill' condition and the 'Elevated bill' condition were calculated for all markers. These differences indicate the displacement of markers.

Elements within the skull

For this analysis the inducing mechanism for cranial kinesis is divided into a number of elements, based on anatomical position. Markers are labelled according to these elements. These elements are the quadrate, the Internal Bar, the Lateral Bar, the Medial Bar and the Dorsal Bar (Fig. 5.1). The quadrate consists only of the *Os quadratum*. The elements in the Internal Bar are the pterygoid, palate and premaxillae. The Lateral Bar is defined by markers along the lateral side of the upper bill and on the jugal bar. The Medial Bar is defined by markers in the *Vomer*, which in Palaeognathae might also transfer forces from the pterygoids onto the upper bill. The Dorsal Bar is the dorsal bar of the upper bill.

It is possible that the externally applied forces to elevate the upper bill result in a deformation of the elements of the PPC, or in a dis-articulation of the joints of the PPC. To test whether deformations or dis-articulations have occurred, distances between markers were calculated in both the 'Closed Bill' and the 'Elevated Bill' condition. If distances between markers within a single element, or between markers across an articulation, have changed between the two conditions, a deformation has occurred. Deformations were tested for all elements (quadrate, Internal Bar, Lateral Bar, Medial Bar and Dorsal Bar) and for the articulations between the quadrate and both the Internal and Lateral Bar. Deformations between conditions were calculated using the same method as for the displacement of the markers.

From the displacement of markers the rotation in the sagittal plane of both the quadrate and the upper bill were determined. Through two markers within each of these elements a line was calculated in both the 'Closed Bill' and 'Elevated Bill' condition. These two lines were projected on a lateral radiogram of the bird, and the intersection of the lines in combination with morphological characters was used to determine the actual point of rotation of the element. From this point of rotation and the displacement of the most distal marker in the element, the final rotation of the element was calculated.

Determination of bending zones

The position of the ventral bending zones in the palaeognathous birds and to a lesser extend in *Calidris* cannot be easily determined from morphological characteristics in radiograms. Therefore the position of these bending zones was calculated by using a four-bar system. The mechanism for the elevation of the upper bill has been described as a four-bar system by Hoese & Westneat (1996). The bars represent the stationary cranium, the quadrate, the PPC, and the caudal side of the upper bill. In a rhynchokinetic skull this last bar is the shortest distance between the flexible zones (*Zonae flexoriae maxillares*) in both dorsal and ventral bar. The lengths of the quadrate and skull bar (see fig. 5.2) and the angle between these bars were measured from radiograms in the rest position. The length of the PPC and the bar formed by the caudal bar of the upper bill were unknown, but kept constant for the 'Closed Bill' and 'Elevated Bill' conditions. For different lengths of the two unknown bars and the measured rotation of the quadrate, the rotation of the upper bill was calculated and the difference with the measured rotation of the upper bill was minimised. The lengths of the elements of the four-bar systems were chosen within he range of the actual morphology. When these four-bar systems were determined the displacement of rotation points were calculated and compared to the measured displacements of markers near the rotation points of the four-bar systems.

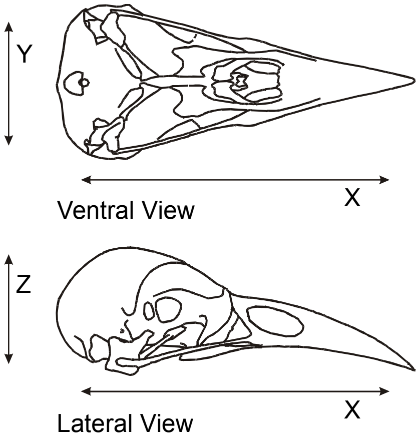


Figure 5.3. Orientation of the axis shown on the skull of the crow (Corvus corone). The X-axis describes the rostral-caudal axis, the Y-axis the lateral-lateral axis, and the Z-axis the dorsal-ventral axis.

Results

The displacement data are represented in three dimensions. The dimensions are according to three axes through the skull. The X-axis describes the rostro-caudal axis of the skull. The Y-axis describes the latero-lateral axis and the Z-axis the dorso-ventral axis (Fig. 5.3).

The Crow (Corvus corone, Table 5.2, Fig. 5.4)

The elevation of the upper bill of the Crow is 6.5 degrees. This rotation angle was based on the values of displacement of the markers in the Dorsal Bar. Marker DB2 is situated closely to the hinge and shows no significant elevation. The DB1 marker, which is situated more rostrally, is elevated 3.45 mm.

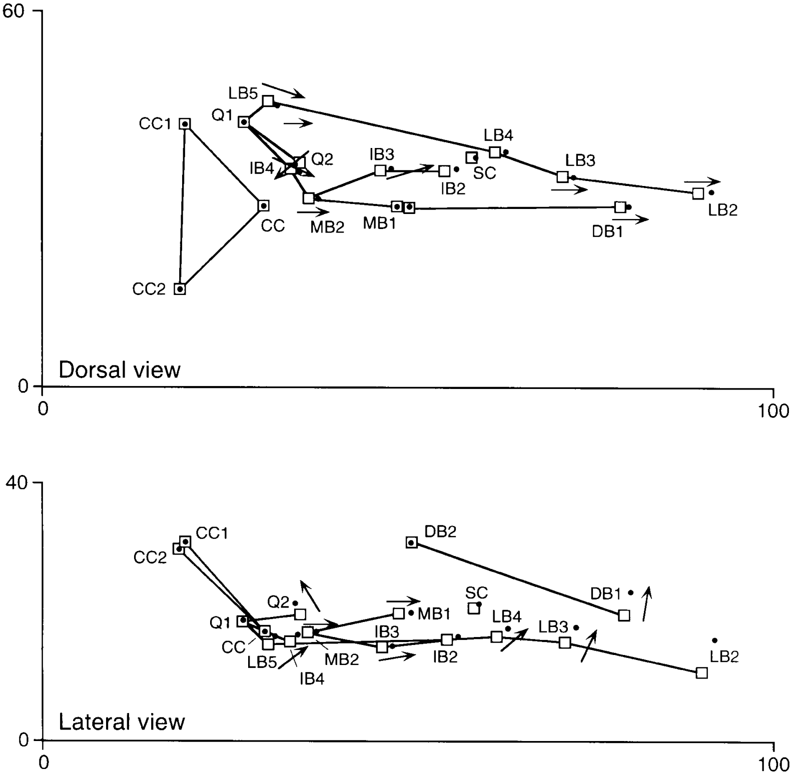


Figure 5.4. Corvus corone. Distribution and relocation of the markers in the X_Z plane (lateral view) and the X-Y plane (dorsal-ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of the markers in the 'Elevated Bill' condition. Letter coding corresponds with the numbers in table 5.1. Drawn lines are schematic indicators of bony elements present in the skull. Distances on the axes are given in millimeters. Arrows indicate the general displacement pattern.

The Lateral Bar markers all show a dorsal and rostral movement which agrees with a forward movement of the jugal bars and a rotation of the upper bill. This movement, although smaller, is also found in the Internal Bar. The Medial Bar shows forward movement and a rotation around the centre of the element, which results in an elevation of the rostral part. Besides the rotation, the Medial Bar also showed a rostral movement of 1.40 mm. The quadrate rotates forward, which results in an upward and slightly backward movement of the *Processus orbitalis* (Q2). The distance between the articulation and marker Q1 was measured in the radiograms (2 mm). This distance and the displacement of marker Q1 were used to calculate the angle around which the quadrate had rotated. This rotation angle was estimated at 10

Table 5.2. Displacement analysis for Corvus corone. Change of marker positions in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated displacement in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

Element	dX	dY	dZ	Dist 3D	s.d.	Dist/s.d.	Moved
LB1							
LB2	1.94	-0.04	5.09	5.45	1.46	3.74	Y
LB3	1.43	0.03	2.38	2.78	1.09	2.55	Y
LB4	1.43	0.07	1.04	1.77	0.93	1.91	Y
LB5	1.21	-0.42	0.97	1.61	0.56	2.89	Y
IB1							
IB2	1.54	0.13	0.75	1.72	0.80	2.14	Y
IB3	1.60	0.24	0.36	1.66	0.67	2.47	Y
IB4	0.84	-0.22	0.81	1.19	0.51	2.34	Y
MB1	1.35	0.05	0.26	1.37	0.68	2.02	Y
MB2	1.44	-0.05	-0.08	1.44	0.52	2.77	Y
DB1	0.69	0.00	3.45	3.52	1.23	2.86	Y
DB2	0.00	0.09	0.03	0.09	0.70	0.13	N
SC	0.84	0.08	0.54	1.01	0.85	1.18	N
CC	0.01	0.00	-0.01	0.02	0.31	0.05	N
C1	0.00	0.00	0.00	0.00	0.35	0.00	N
C2	0.00	-0.04	-0.01	0.04	0.35	0.12	N
Q1	0.18	-0.09	0.26	0.33	0.49	0.67	N
Q2	-0.73	-0.68	1.82	2.08	0.48	4.35	Y
Q3							

degrees not including inward rotation. The cranial markers showed no change in position, which indicated no movement in these elements. Similarly, no deformations as a result of the external opening forces on the upper bill were observed within elements.

The Red Knot (Calidris canutus, Table 5.3, Fig. 5.5)

The rotation of the moveable part of the upper bill in the Red Knot was approximately 10 degrees. This angle was calculated from the displacement of markers LB2 and LB3 in the

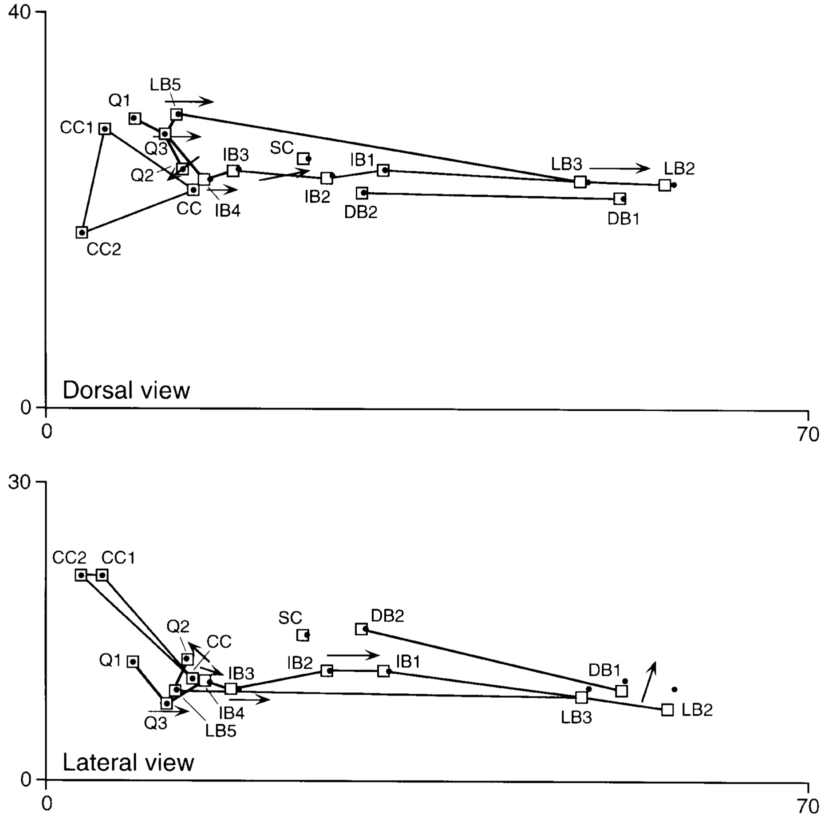


Figure 5.5. Calidris canutus. Distribution and relocation of the markers in the X_Z plane (lateral view) and the X-Y plane (dorsal-ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of the markers in the 'Elevated Bill' condition. Letter coding corresponds with the numbers in table 5.1. Drawn lines are schematic indicators of bony elements present in the skull. Distances on the axes are given in millimeters. Arrows indicate the general displacement pattern.

Table 5.3. Displacement analysis for Calidris canutus. Change of visible points in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated displacement in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

Element	dX	dY	dZ	Dist 3D	s.d.	Dist/s.d.	Moved
LB1							
LB2	0.61	0.06	2.21	2.29	1.16	1.97	Y
LB3	0.54	0.04	0.81	0.98	0.97	1.01	N
LB4							
LB5	0.26	-0.14	0.24	0.38	0.54	0.69	N
IB1	0.42	0.10	0.06	0.44	0.56	0.78	N
IB2	0.42	0.09	-0.05	0.43	0.48	0.90	N
IB3	0.41	0.06	-0.03	0.41	0.44	0.94	N
IB4	0.34	0.04	-0.05	0.35	0.43	0.81	N
MB1							
MB2							
DB1	0.27	0.00	1.10	1.13	1.07	1.05	N
DB2	0.04	-0.03	-0.05	0.07	0.56	0.13	N
SC	0.37	0.00	0.00	0.37	0.30	1.20	N
CC	0.02	-0.02	-0.02	0.04	0.30	0.13	N
C1	0.00	0.00	0.00	0.00	0.33	0.00	N
C2	0.00	0.01	0.00	0.01	0.56	0.02	N
Q1	-0.02	0.02	-0.04	0.05	0.55	0.09	N
Q2	-0.25	-0.22	0.23	0.40	0.41	0.98	N
Q3	0.31	-0.08	0.12	0.35	0.55	0.63	N

Lateral Bar. The rotation of the quadrate, based on the rotation of the line through markers Q3 and Q1 is estimated to be 9 degrees. Marker LB2 is the only marker that has moved significantly. Although differences in other markers are not significant, due to the small size of the bird and thus of the displacements, the pattern of movement is very similar to that of the crow. Markers in the Lateral Bar all show a rostral and a slightly dorsal movement. The Internal Bar shows only a rostral movement and the rostral marker in the Dorsal Bar moves slightly dorsally and rostrally. There were no significant changes in distances between and within elements when conditions were compared.

The Rhea (Rhea americana, Table 5.4, Fig. 5.6)

The rotation of the upper bill was calculated from the displacements in the Dorsal Bar (DB1, DB2). The rotation angle was approximately 8 degrees. The quadrate rotated 8 degrees in rostro-dorsal direction and also rotated slightly to medial.

Marker DB2 showed no displacement, Marker DB1 was rotated upward and pulled slightly forward during opening. Markers in the Lateral Bar showed larger displacement in the rostral direction than the markers in the Dorsal Bar. The dorsal displacement of the markers in the

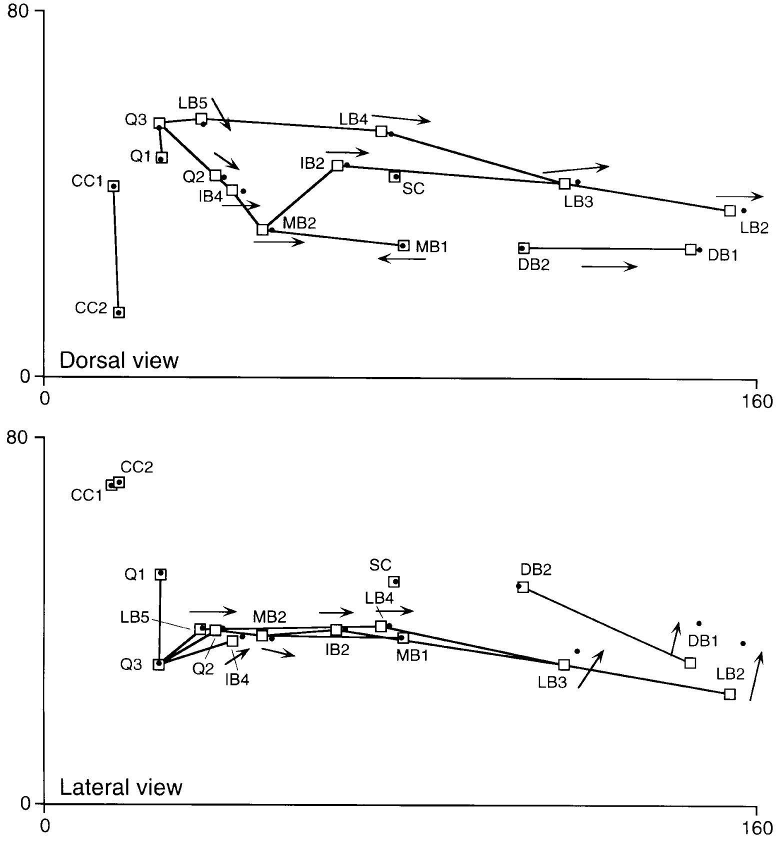


Figure 5.6. Rhea americana. Distribution and relocation of the markers in the X-Z plane (Lateral View) and the X-Y plane (Dorso-Ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of markers in the 'Elevated Bill' condition. Letter codings correspond with the numbers in table 5.1. Drawn lines are schematic indicators of bony elements present in the skull. Distances on the axes are given in millimeters. Arrows indicate the general displacement pattern.

Table 5.4. Displacement analysis for Rhea americana. Change of visible points in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated displacement in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

Element	dX	dY	dZ	Dist 3D	s.d.	Dist/s.d.	Moved
LB1							
LB2	3.01	-0.09	10.92	11.32	0.92	12.30	Y
LB3	2.79	0.15	2.77	3.94	0.68	5.79	Y
LB4	2.15	-0.30	0.23	2.18	0.52	4.19	Y
LB5	0.44	-0.84	0.35	1.01	0.57	1.77	Y
IB1							
IB2	2.17	-0.44	-0.05	2.21	0.49	4.52	Y
IB3							
IB4	2.06	-0.02	0.71	2.18	0.50	4.38	Y
MB1							
MB2	2.30	0.13	-0.39	2.33	0.49	4.76	Y
DB1	1.71	0.00	8.78	8.95	0.88	10.11	Y
DB2	-0.78	0.28	-0.04	0.82	0.63	1.31	N
SC	0.47	-0.11	-0.15	0.51	0.32	1.58	N
CC							
C1	0.00	0.00	0.00	0.00	0.39	0.00	N
C2	0.00	-0.04	0.00	0.04	0.38	0.11	N
Q1	0.12	-0.26	0.13	0.32	0.50	0.64	N
Q2	1.56	-0.28	0.47	1.66	0.50	3.30	Y
Q3							

Lateral Bar was very large for the most rostral markers, but smaller for the more caudal markers. The Internal Bar showed mainly rostral displacement. The marker in the pterygoid (IB4) was also slightly displaced in dorsal direction. The cranial markers (C1, C2) showed no displacement at all and the marker in the ectethmoid bone (SC) only slight non-significant displacement in the dorsal direction.

Deformations were found in the Lateral Bar as result of the elevation of the upper bill in the Rhea. Within the Lateral Bar the distance between markers LB3 and LB5 (-1.99 mm), and between LB4 and LB5 (-1.67 mm) decreased as a result of upward bending in the Lateral Bar. No other deformations were observed in either elements or articulations.

The Emu (Dromaius novaehollandiae, Table 5.5, Fig. 5.7)

The rotation of the upper bill was determined from the displacement of the markers in the Dorsal Bar. The upper bill was rotated 4 degrees upward. The Quadrate was rotated 4 degrees in the rostro-dorsal direction and showed also a large medial displacement of the more distal markers (Q2, Q3).

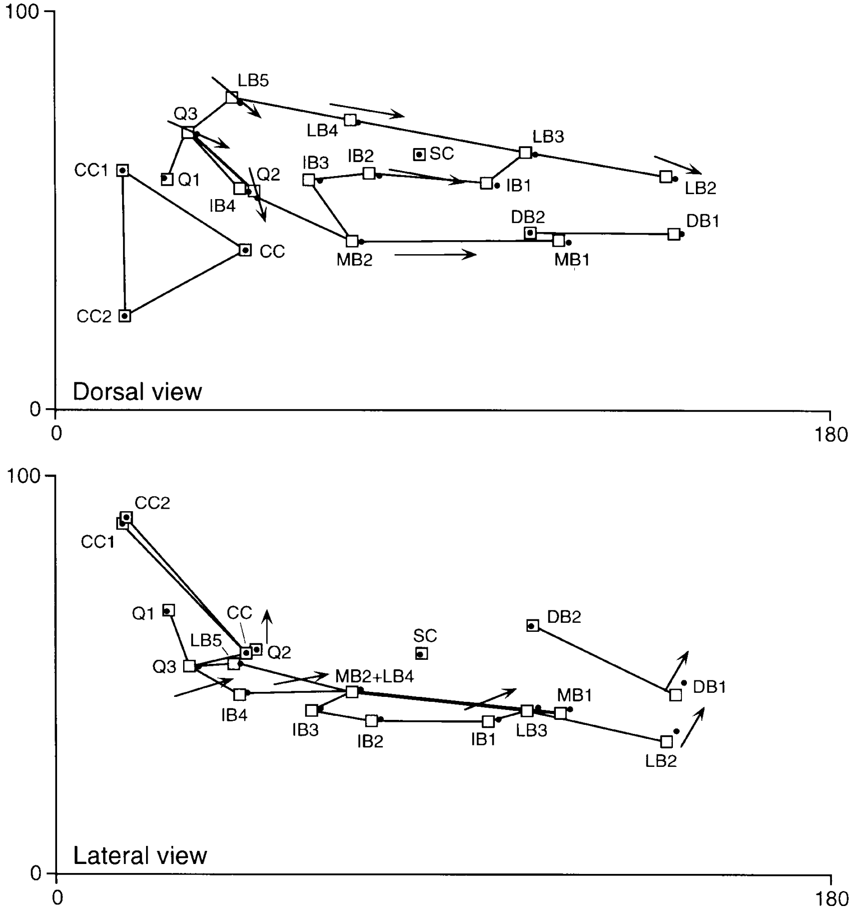


Figure 5.7. Dromaius novaehollandiae. Distribution and relocation of the markers in the X-Z plane (Lateral view) and the X-Y plane (Dorso-Ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of markers in the 'Elevated Bill' condition. Letter coding correspond with the letters in table 5.1 Drawn lines are schematically indicators of bony elements present in the skull. Distances on the axes are given in millimeters. Arrows indicate the general displacement pattern.

Table 5.5. Displacement analysis for Dromaius novaehollandia. Change of visible points in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated displacement in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

Element	dX	dY	dZ	Dist 3D	s.d.	Dist/s.d.	Moved
LB1							
LB2	2.40	-0.48	2.58	3.56	1.32	2.69	Y
LB3	2.07	-0.39	1.08	2.36	1.02	2.31	Y
LB4	1.93	-0.85	0.79	2.25	0.70	3.24	Y
LB5	1.79	-1.13	0.28	2.13	0.61	3.49	Y
IB1	2.16	-0.37	0.82	2.34	0.93	2.52	Y
IB2	2.16	-0.58	0.58	2.31	0.72	3.21	Y
IB3	2.11	-0.43	0.36	2.19	0.62	3.52	Y
IB4	1.56	-0.88	0.17	1.80	0.53	3.41	Y
MB1	1.97	-0.43	1.33	2.42	1.06	2.28	Y
MB2	1.81	-0.18	0.26	1.84	0.63	2.89	Y
DB1	1.21	0.00	3.26	3.48	1.32	2.63	Y
DB2	-0.18	0.07	0.23	0.30	0.97	0.31	N
SC	0.10	-0.09	0.14	0.19	0.78	0.25	N
CC	-0.08	-0.03	0.09	0.12	0.30	0.42	N
C1	0.00	0.00	0.00	0.00	0.30	0.00	N
C2	0.00	0.09	-0.01	0.09	0.29	0.29	N
Q1	-0.15	0.16	-0.04	0.22	0.43	0.51	N
Q2	0.15	-1.42	1.22	1.88	0.48	3.93	Y
Q3	1.32	-0.57	-0.05	1.44	0.55	2.64	Y

The markers in the Lateral Bar showed a similar displacement pattern as in the Rhea. The markers of the Lateral Bar moved rostral and dorsal. The rostral movement is almost constant throughout the bar while the dorsal movement is larger in the more dorsal markers. The Internal Bar showed mainly rostral movement, over a distance similar to that of the Lateral Bar. Slight dorsal movements were also measured but these were very small. The same displacement pattern was also found in the Internal Bar and Medial Bar. In both bars the rostral markers showed a slightly larger dorsal displacement than the more caudal markers. None of the cranial markers showed any displacement. No significant deformations were found in elements or articulations as a result of the opening of the upper bill.

The Ostrich (Struthio camelus, Table 5.6, Fig. 5.8)

The upper bill of the Ostrich was elevated 8 degrees. This angle was calculated from the displacement of the most rostral markers of the Lateral Bar (LB1, LB2). The quadrate rotated 5 degrees rostro-dorsal around the Processus oticus and showed a medial rotation also.

The Lateral Bar, Medial Bar and Internal Bar all show a rostral displacement. The more rostral markers also showed a dorsal displacement. None of the cranial markers showed any displacement. No internal deformations within elements or articulations were observed.

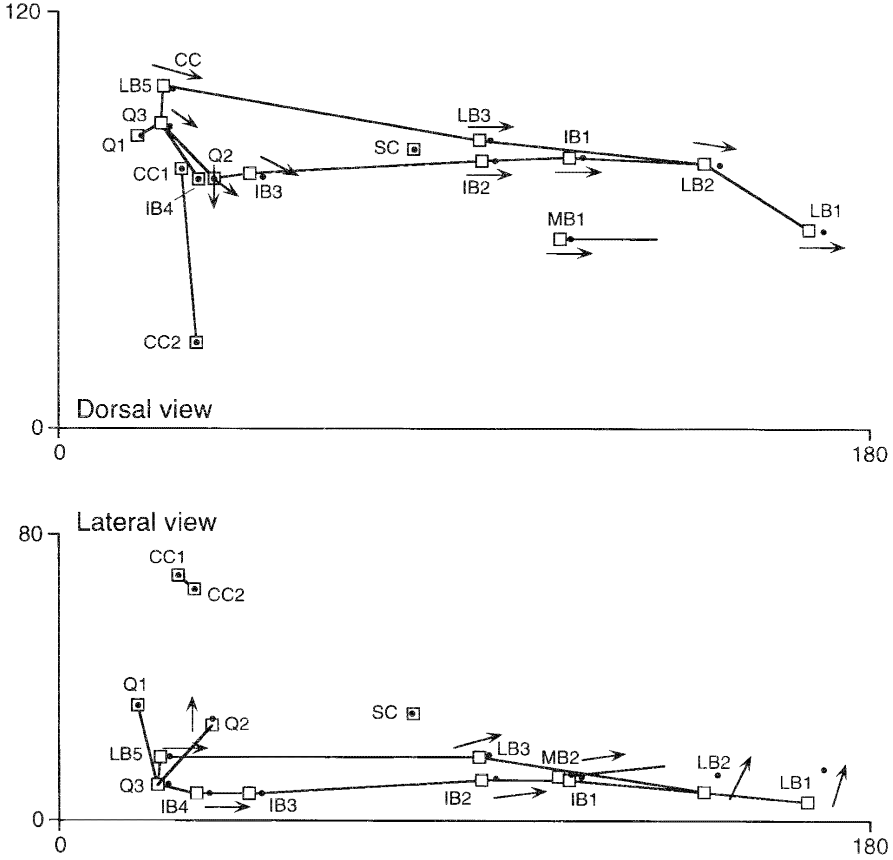


Figure 5.8. Struthio camelus. Distribution and relocation of the markers in the X-Z plane (Lateral view) and the X-Y plane (Dorso-Ventral view). Squares indicate the position of markers in the 'Closed Bill' condition, dots indicate the position of markers in the 'Elevated Bill' condition. Letter coding correspond with letters in table 5.1. Drawn lines are schematical indicators of bony elements present in the skull. Distances on the axes are giver in millimeters. Arrows indicate the general displacement pattern.

Table 5.6. Displacement analysis for Struthio camelus. Change of visible points in the experimental object in three directions (all in mm): dX: in rostro-caudal direction, dY: in latero-medial direction, dZ: in dorso-ventral direction. Column s.d. gives the standard deviation of the calculated displacement in mm. When the distance of relocation between the two conditions divided by its standard deviation is smaller than the critical value of the normal distribution (1.65, p<0.05) the marker is considered stationary. Movements are expressed as: No (N) if the marker has remained stable and Yes (Y) if the marker has moved.

Element	dX	dY	dZ	Dist 3D	s.d.	Dist/s.d.	Moved
LB1	3.11	-0.60	9.19	9.72	0.98	9.90	Y
LB2	3.00	-0.31	4.84	5.70	0.81	7.00	Y
LB3	2.49	-0.14	0.54	2.55	0.56	4.57	Y
LB4							
LB5	1.94	-1.13	-0.22	2.26	0.64	3.55	Y
IB1	2.73	-0.04	1.23	2.99	0.65	4.60	Y
IB2	2.65	-0.12	0.45	2.69	0.57	4.69	Y
IB3	2.63	-0.94	0.15	2.80	0.55	5.11	Y
IB4	2.70	-1.07	0.11	2.91	0.57	5.09	Y
MB1	2.62	0.00	0.25	2.63	0.66	3.99	Y
MB2							
DB1							
DB2							
SC	0.25	0.11	-0.19	0.34	0.34	1.00	N
CC							
C1	0.00	0.00	0.00	0.00	0.42	0.00	N
C2	0.00	-0.06	0.00	0.06	0.39	0.16	N
Q1	0.24	-0.33	-0.08	0.42	0.58	0.72	N
Q2	0.21	-1.17	1.64	2.03	0.49	4.12	Y
Q3	2.05	-1.39	0.13	2.48	0.63	3.95	Y

The Four-Bar systems

For all skulls a two-dimensional four-bar system was determined that describes the rotation of the Quadrate and Upper Bill, as well as the displacement of the markers. These four-bar systems were used to determine the position of the flexible ventral zones in the upper bill. The lengths of the elements for each species are described in table 5.7 and schematically represented in figure 5.9. Estimated and calculated displacements of markers are given in table 5.8. For all species the calculated displacements are very similar to the displacements measured using the stereo-photogrammetry method. This indicates that the four-bar systems

Table 5.7. Estimated four-bar systems describing the skull (Fig. 5.9). Skull: the stationary part of the four bar system, Quadrate the length of the quadrate, PPC: the length of the PPC and Bill the distance between the rotation points in the dorsal and ventral bar of the upper bill. Ang. Q is the angle between the 'Skull'- bar and the Quadrate, Ang. B. the angle between the Skull-bar and the Bill-bar. The dAng. Q and dAng. B represent the change of the Quadrate Angle and Bill Angle between the closed and opened condition.

Species	Skull	Quadrate	PPC	Bill	Ang. Q.	Ang. B.	dAng. Q	dAng. B.
Corvus	31.5	10.0	29.0	13.0	68	86	10	6.5
Calidris	46.0	5.0	40.0	3.0	32	126	9	10
Rhea	89.0	16.0	87.0	15.0	55	61.5	8	8
Dromaius	94.0	19.0	98.0	22.0	43	35.5	4	4
Struthio	107.0	31.0	111.0	24.0	67	49.5	5	8

provide a good description of the displacement in the skull after elevation of the upper bill. The position of the flexible zones in the ventral bars of the upper bill were estimated to be at the following positions from the bill tip: *Struthio*: 36 mm, *Rhea*: 47 mm, *Dromaius*: 37 mm, *Calidris*: 12 mm.

Discussion

Avian cranial kinesis is controlled by several bony elements. Although the system has been described qualitatively for prokinetic birds (Bock, 1964) the actual displacements of the elements in the jaw apparatus have only been measured in two dimensions in a single species (van Gennip & Berkhoudt, 1992). Our three-dimensional analysis of displacement patterns of the bony elements in the skull shows that the general displacement pattern is indeed valid for both prokinetic and rhynchokinetic birds. The elevation of the upper bill in birds is initiated by a rostro-caudal rotation of the quadrate. This rotation results in a rostral displacement of both the jugal arc and the PPC. These two bars press against the premaxilla, which results in elevation of either the complete upper bill (prokinesis and extensive rhynchokinesis), or only the rostral

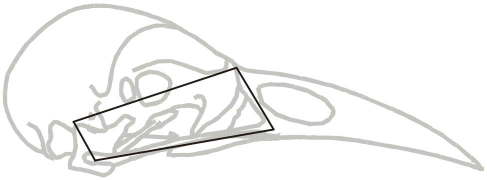
Table 5.8. Calculated and measured displacement of markers closest to the moveable connections of the four-bar systems. Markers indicate the marker used, dXZ M the measured displacement in the XZ-plain, dXZ C the calculated displacement in the XZ-plain.

Species	Caudal Marker	dXZ M	dXZ C	Rostral Marker	dXZ M	dXZ C
Corvus	IB4	0.9	1.2	SC	0.9	0.9
Calidris	IB4	0.3	0.8	LB3	1.0	0.5
Rhea	IB4	2.2	2.2	LB4	2.2	2.1
Dromaius	IB4	1.6	1.3	LB3	2.3	1.5
Struthio	IB4	2.7	2.7	IB1	3.0	3.3

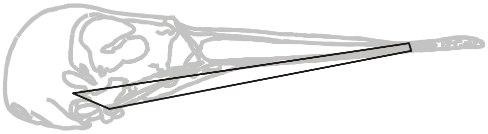
part of the upper bill (distal rhynchokinesis).

Our kinematic analysis shows that these displacement patterns are valid for all types of cranial kinesis. This indicates that no differences in the PPC are to be expected on the basis of the type of cranial kinesis alone. This is in contrast with several hypotheses about the origin of the palaeognathous PPC. The palaeognathous PPC is always linked to the central kinesis found in these birds (Bock, 1963; Hofer, 1954; Simonetta, 1960). An analysis of *Calidris*, a bird with a distal rhynchokinetic bill used for probing, shows that the PPC is very similar to that of prokinetic birds. The adaptations found in this species are mainly due to the functional demands of probing behaviour and not due to the type of cranial kinesis (Gerritsen, 1988; Chapter 3). The present analysis confirms that the type of cranial kinesis does not pose specific functional demands on the PPC.

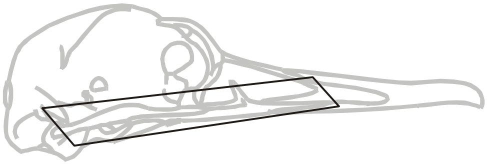
The main difference between the various types of cranial kinesis is the position of the rotation points in the upper bill (see Zusi, 1984). The prokinetic crow has a very clear rotation point of the upper bill at the nasal-frontal hinge. All other birds in this study have bending zones instead of a true rotation point. The position of these bending zones can be determined based on a comparison of elevated and non-elevated skulls. In *Calidris* these bending zones can also be morphologically identified from the thickness of the bars. In the Palaeognathae, however, there seems to be no clear morphological characters that indicate the position of these bending zones. In *Calidris* our predicted bending point coincides with the thinnest part in the elements of the upper bill (Gerritsen, 1988; see also chapters 3 and 6).



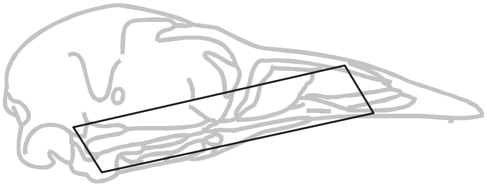
Corvus corone



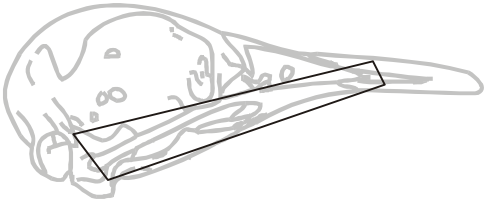
Calidris canutus



Rhea americana



Dromaius novaehollandiae



Struthio camelus

Figure 5.9. Four bar systems projected onto the skulls in lateral view. Different skulls are not drawn to scale.

The four-bar systems show that the movement of the PPC and the upper bill can be predicted with a two dimensional model. Very few three-dimensional models have been developed. The three-dimensional model of van Gennip & Berkhoudt (1992) shows that lateral/medial movement of the quadrate is of great importance in the Pigeon. During grasps, pigeons open both jaws, but during intra-oral transport only the mandible is depressed. The medial quadrate movement uncouples upper and lower jaw movement, which they assume to be linked by the postorbital ligament. Our analysis shows that during upper bill rotation the displacement of the quadrate in the lateral/medial plain is, except for the crow, very limited. However, the quadrate does show rotation along its longitudinal axis, which mainly effects the position of the *Processus orbitalis quadrati*. A rotation around this axis will not affect the forward motion of the quadrate.

In recent papers (Zweers *et al.*, 1997; Zweers & Vanden Berge, 1997b) the PPC is considered one of the elements that had a major impact on the early evolution of trophic systems in birds. In their hypothesis the detachment of the PPC from the cranium was a key-innovation, which resulted in a wide trophic radiation in birds. Although this argument in itself might proof to be true, they also propose that the detachment of the PPC resulted in three different anatomical designs of the PPC: a primary rhynchokinetic (*Hesperornis*), a palaeognathous rhynchokinetic (Palaeognathae), and a prokinetic design. Later in evolution the latter gave rise to the Charadriiform rhynchokinesis. Our kinematic analysis of the PPC shows however no major differences in the movement pattern of the elements of the PPC in modern birds. Although parallel evolution cannot be excluded, both the movement patterns and morphology (McDowell, 1948; Gussekloo & Zweers, 1999) are so similar within modern birds, compared to other modern (reptilian) taxa, that a polyphyletic origin of the avian kinetic skull is very unlikely. Based on the movement patterns we conclude that all kinetic types described in this study must have evolved from a single kinetic ancestor.

Acknowledgements

We like to thank M. Heijmans for carefully constructing the object and calibration frame.

CHAPTER 6

CRANIAL KINESIS IN PALAEOGNATHOUS BIRDS

Summary

In palaeognathous birds the morphology of the Pterygoid-Palatinum Complex (PPC) is remarkably different from the PPC of neognathous birds. Palaeognathae are also believed to possess a specific type of cranial kinesis known as central rhynchokinesis. We experimentally determined bending forces opposing elevation of the upper bill and used a mathematical model to calculate the opening forces that can be produced by the Palaeognathae. It was found that muscle forces are more than sufficient to overcome bending forces and to elevate the upper bill. The large pterygoid-muscles contribute only slightly to the total biting force, but may resist external opening forces on the upper bill. No clear bending zones are present in the upper bill, and if bending occurs it will be over the total length of the upper bill. We conclude that the palaeognathous upper bill and PPC are designed to resist external forces that elevate the upper bill.

Introduction

Cranial kinesis is an important character of the feeding behaviour of birds and, to a lesser extent, of reptiles. In birds cranial kinesis is characterised by the ability to move the upper bill or a part thereof. Most birds are able to move the whole upper bill, a situation known as prokinesis (Bock, 1964). The bending zone between the skull and moveable upper bill is situated at the nasal-frontal hinge. Other bending zones are situated on the transition between the palate and the upper bill and between the jugal bar and the upper bill. The prokinetic upper bill as a whole contains no flexible zones and is rigid. This type of cranial kinesis is considered the most basic form within modern birds (Bock, 1964). Several other types of cranial kinesis can be distinguished, based on different positions of the bending zones. The second most common type is the rhynchokinetic configuration. In this type the bending zones are positioned further rostrally within the upper bill. This means that in these species only a small portion of the upper bill is moveable and bending zones are present in both the dorsal and ventral bars. Rhynchokinesis also has consequences for the rest of the configuration of the skull. To make the movement of the rostral part of the upper bill possible, the movements of the dorsal and ventral bars have to be uncoupled. This uncoupling is achieved by a schizorhinal nostril (Fig. 1.3), which means that the dorsal attachment of the lateral bar, connecting the dorsal and ventral bar, is positioned caudal to the nasal-frontal hinge (Zusi, 1984). Although the main division in types of cranial kinesis is between prokinetic and rhynchokinetic skulls, a number of sub-divisions is recognised within the rhynchokinetic type, which are characterised by different positions of the flexible zones (Zusi, 1984). In the proximal rhynchokinetic type the bending zones are just rostral to the nasal-frontal area, in central rhynchokinesis the bending zones are positioned in the middle of the upper bill, and in distal rhynchokinesis the bending zones are just caudal of the rostrum maxillae. One subtype, double rhynchokinesis, has two bending zones and is a combination of proximal and distal rhynchokinesis. Finally, extensive rhynchokinesis is characterised by a flexible zone that extends over almost the entire bill. In all these types a schizorhinal nostril is present, except in central or palaeognathous rhynchokinesis. In the latter subtype uncoupling of the dorsal and ventral bar is achieved by a gap in the lateral bar of the upper bill. It is remarkable that this type of rhynchokinesis is found in one avian taxon only: the Palaeognathae.

The Palaeognathae is one of the taxa of the most basal sub-division within the taxon Aves (Cracraft, 1974; Sibley & Ahlquist, 1990). This basal subdivision divides the taxon Aves into the Palaeognathae and the Neognathae. The Palaeognathae is a small taxon, approximately 60 species, versus 8000 species of Neognathae. Only a few, mainly cranial, morphological characters distinguish the Palaeognathae from the Neognathae (Olson, 1985). The most important character is the presence of the palaeognathous Pterygoid-Palate Complex (PPC; Gussekloo & Zweers, 1999), which consists of the pterygoid, palate and the vomer. In Palaeognathae the PPC is rigid and lacks the flexible articulations between elements found in Neognathae (McDowell, 1948; Bock 1963). In neognathous birds this complex, in combination with the quadrate, has a clear function in the movement of the upper bill. The movement of the

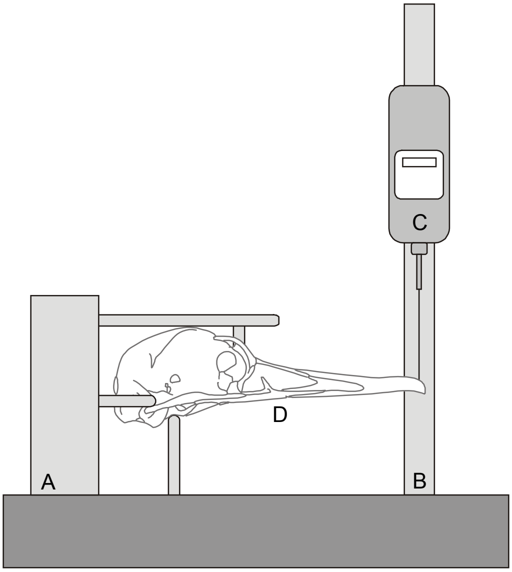


Figure 6.1. Experimental set-up in lateral view. A. Fixation rods, B. Step motor, C. Force transducer, D. Experimental object.

upper bill is caused by a rotation of the quadrate, which is transferred to the upper bill via the PPC (Bock, 1964). It has always been assumed that the PPC in the Palaeognathae serves the same function, and that central rhynchokinesis is related to the special morphology of the PPC (Bock, 1963; Hofer, 1954; Simonetta, 1960).

In this paper we test whether the skulls of the Palaeognathae are rhynchokinetic on the basis of a number of morphological characters typical for rhynchokinesis and which are present in other rhynchokinetic birds, such as Charadriiformes (Zusi, 1984; Chapter 3). These characters are the presence of a uncoupling of the dorsal and ventral bar, and the presence of clear bending zones in both the ventral and dorsal bar of the upper bill. We also tested whether muscle forces are sufficient for rhynchokinetic movement of the upper bill. Earlier analyses of cranial kinesis of the Palaeognathae have been done by manipulating osteological specimens and were mainly qualitative (Bock, 1963; Hofer, 1954; Simonetta, 1960). In this study we try to quantify both the presence and location of bending zones.

Materials and Methods

Three species of palaeognathous birds were used for the analysis: the Ostrich (*Struthio camelus*) from Africa, The Greater Rhea (*Rhea americana*) from South- America and the Emu (*Dromaius novaehollandiae*) from Australia. Two specimens of both the Emu and Rhea and three specimens of the Ostrich were used. All specimens were heads of deceased birds, which had been stored at a temperature of –20 ^{o}C. Before the experiments the skin was removed to facilitate fixation in the experimental set-up (see below). All other parts of the skull were kept intact.

The forces necessary to elevate and depress the upper bill were determined in an experimental set-up (Fig. 6.1) in which the upper bill was depressed or elevated and reaction forces were measured using a force-transducer (Aikoh). The head was fixed with screws on each side, and a bar was attached to the skull to prevent dorso-ventral rotation. The force transducer was attached to the tip of the upper bill. For elevation a small hook was attached to the upper bill and the force-transducer was slowly moved upward by a step motor. For depression a blunt bar was used to push against the upper bill while the force-transducer moved downward. The force-transducer moved with a speed of 5 mm/sec while forces and elevation were recorded continuously. All experiments were performed at room temperature.

The specimens were then dissected and of each muscle the weight and mean fibre length was determined. Fibre-lengths were determined using the fibre-dissecting technique of Gaunt & Gans (1990). An estimate of maximal muscle forces was made from the physiological cross section of the muscle according to the formula:

$$F_{\max} = \frac{m}{l \cdot \rho} \cdot M_c$$

with: $F_{\max}$ = maximal muscle force (N); m = muscle mass (kg); l = mean fibre length (m); ρ = muscle density (1000 kg.m^{-3}); M_c = muscle stress constant ($330\text{x}10^3$ N.m^{-2}; Hildebrand *et al.*, 1985).

The estimated maximal muscle forces were used to calculate the force that can be asserted on the upper bill, and the maximal biting force. These forces were calculated with two-dimensional models, in which the skull is represented as a four-bar system. The bars represented the quadrate, the PPC, the caudal side of the moveable part of the upper bill, and the stationary skull between the rotation point of the quadrate and the flexible zone in the dorsal bar of the upper bill. The quadrate bar had the length and orientation of the quadrate. The PPC bar had the length and the orientation of the line between the ventral part of the quadrate bar and the centre of the bending zone in the ventral bar of the upper bill. The PPC bar describes roughly the orientation of the PPC. The Bill bar is defined by the line between the centres of the bending zones in the ventral and dorsal bar. Finally the stationary skull bar is described by the line between the dorsal end of the quadrate bar and the centre of the bending zone in the dorsal bar of the upper bill. The moveable part of the upper bill is added to the Bill bar. This part is defined by both ends of the bill bar and the bill tip and moves as a whole with the Bill bar. All articulations included in the model are considered to be rotation points without friction or other opposing forces and one degree of freedom (rotation). The position of the flexible zones in the upper bill was measured in lateral radiograms and in video-recordings of the force measurement experiments. The lower jaw articulates around the ventral point of the quadrate and is in its rest position in all calculations. All forces were calculated under the assumption of static equilibrium. The lengths of the elements of the four-bar system were measured in lateral radiograms. Orientations of muscles were estimated in lateral radiograms from the known position of origo and insertio of the muscles. Schematic drawings of the models used for the different species are given in figures 6.2.

A number of forces were calculated using the models. The elevation force on the upper bill, defined as the necessary force on the upper bill tip to balance the maximal force of the elevator muscle of the upper bill (*Musculus protractor pterygoideus et quadrati*), was calculated and compared to measured elevation forces. The contribution of the adductor muscles to biting forces of the lower jaw was calculated at the lower bill tip as the necessary force to cancel the maximal force of the *Musculus adductor externus* complex. Finally total biting forces were calculated for a food-item of 10 mm at the bill tip. This was done using an adapted version of the model of the avian jaw apparatus of Bout (*in prep.*) and included the four-bar system, the lower jaw and the reaction force at the *Processus basipterygoideus*. This processus is only found in Palaeognathae (McDowell, 1948) and blocks in the rest position and in combination with the *Processus zygomaticus* caudal movement of the PPC. For the calculation of the maximal biting force the upper bill elevation was varied between 0 and 10 degrees and maximum forces of the *Musculus adductor mandibulae externus*, *Musculus pterygoideus* and Adductor muscles on the quadrate were used.

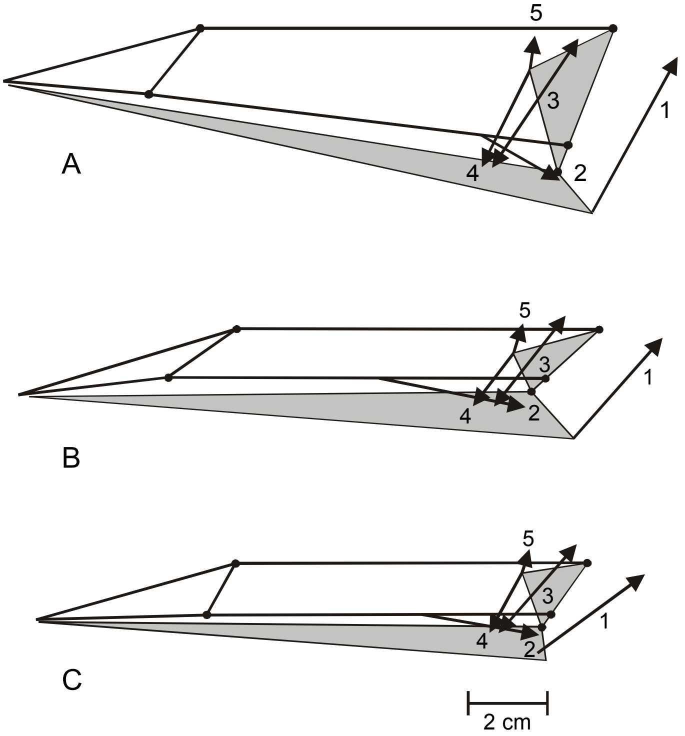


Figure 6.2. Schematic representation of the skull of three species of Paleognathae. A. Ostrich (Struthio camelus), B. Emu (Dromaius novaehollandiae), C. Rhea (Rhea americana). Arrows indicate the line of action and the length of the muscles: 1. Musculus depressor mandibulae, 2. Musculus pterygoideus, 3. Musculus adductor mandibulae externus, 4. Adductor muscle complex of the quadrate, 5. Musculus protractor pterygoidei et quadrati. Gray areas represent the quadrate and the lower bill. Circles represent rotation points.

Table 6.1. Mass, Fibre length and Maximal muscles forces from the 5 most important muscle complexes in the skull. The Adductor mandibulae complex consists of two parts with unequal fibre-lengths, but with similar work-lines. The total force of these parts is combined as 'Total Max. Force'. Mean values of both mass and fibre lengths are given.

Struthio	**Adductor Mandibulae**	**Pterygoid**	**Depressor Mandibulae**	**Protractor Quadratum**	**Adductor Quadratum**
Mass (g)	4.2 ± 0.9 (6) 2.6 ± 0.2 (6)	6.8 ± 0.7 (6)	6.4 ± 0.9 (6)	1.0 ± 0.2 (6)	2.3 ± 0.1 (6)
Fibre Length (mm)	16.4 ± 4.6 (6) 14.8 ± 5.3 (6)	16.6 ± 4.2 (6)	44.0 ± 11.4 (6)	7.6 ± 2.4 (5)	12.4 ± 4.7 (6)
Maximal Force (N)	96 ± 48 (6) 68 ± 31 (6)	147 ± 57 (6)	51.5 ± 15.7 (6)	47 ± 24 (5)	72 ± 36 (6)
Total Max. Force (N)	164				

Dromaius	**Adductor Mandibulae**	**Pterygoid**	**Depressor Mandibulae**	**Protractor Quadratum**	**Adductor Quadratum**
Mass (g)	1.9 ± 0.1 (4) 1.1 ± 0.0 (4)	4.0 ± 0.1 (4)	3.8 ± 0.0 (4)	1.2 ± 0.0 (4)	1.8 ± 0.1 (4)
Fibre Length (mm)	16.0 ± 1.2 (4) 12.5 ± 0.5 (4)	18.8 ± 0.6 (4)	24.4 ± 0.3 (4)	9.0 ± 0.6 (4)	17.5 ± 0.4 (4)
Maximal Force (N)	40 ± 3.9 (4) 28 ± 0.0 (4)	70 ± 0.5 (4)	51 ± 0.1 (4)	43 ± 3.0 (4)	35 ± 0.5 (4)
Total Max. Force (N)	68				

Rhea	**Adductor Mandibulae**	**Pterygoid**	**Depressor Mandibulae**	**Protractor Quadratum**	**Adductor Quadratum**
Mass (g)	2.2 ± 0.2 (4) 1.4 ± 0.1 (4)	3.2 ± 0.4 (4)	2.0 ± 0.2 (4)	0.5 ± 0.3 (2)	0.6 ± 0.4 (4)
Fibre Length (mm)	9.1 ± 0.3 (4) 4.8 ± 1.1 (4)	8.7 ± 0.9 (4)	28.1 ± 2.8 (4)	5.4 ± 1.2 (4)	5.8 ± 1.2 (4)
Maximal Force (N)	80 ± 8.5 (4) 95 ± 25 (4)	123 ± 24 (4)	23.9 ± 4.7 (4)	36 ± 13 (2)	32 ± 15 (4)
Total Max. Force (N)	175				

To test whether flexible zones are present in the upper bill of the palaeognathous birds the thickness of the ventral and dorsal elements was measured. The measurements were performed on the specimens used for the force measurements and some additional osteological specimens. The thickness of both ventral bars and the dorsal bar were measured using a digital calliper rule (Sylvac, accuracy 0.01 mm). One Ostrich bill was used to make transverse sections. The sections were stained according to the van Giesson method (Brodal *et al.*, 1978) and used to measure the thickness of the dorsal and ventral bars more accurately under a dissection microscope. For comparison, the thickness of the dorsal and ventral bars were measured in a similar way in transverse sections of the bills of the Purple Sandpiper (*Calidris maritima*) and the Sanderling (*Calidris alba*), which have a distal rhynchokinetic skull. All thickness measurements were scaled to the head-width, measured at the quadrato-jugal articulation, to eliminate size effects. In the analysis bending zones were only considered effective when they were present caudal to the *Rostrum maxillae* and rostral to the nasal process of the maxillae. In some species the *Rostrum parasphenoidale* extents beyond the caudal limit of the upper bill. In these cases the most rostral point of the *Rostrum parasphenoidale* is taken as the caudal border for the position of an effective bending zone. Outside these borders the bill is unable to bend because both the *Rostrum maxillae* and *the Rostrum parasphenoidale* are very thick and rigid. The position of the nasal process is chosen as the caudal border of the bill.

Table 6.2. Lengths of the elements of the four bar systems. For description of the elements see text. Angle Q and Angle B indicate the angle of the quadrate and of the caudal side of the upper bill relative to the skull bar.

Species	**Skull**	**Quadrate**	**PPC**	**Bill**	**Angle Q.**	**Angle B.**
Struthio	107.0	31.0	111.0	24.0	67	49.5
Dromaius	94.0	19.0	98.0	22.0	43	35.5
Rhea	89.0	16.0	87.0	15.0	55	61.5

Results

Muscle Forces

The cranial muscles acting in feeding behaviour can be divided into 5 major functional groups. These groups are: 1) closer of the lower bill (*Musculus adductor mandibulae externus* complex), 2) closer of the upper bill (*Musculus pterygoideus* complex), 3) closer of both upper and lower bill (adductor complex of the quadrate), 4) opener of the lower bill (*Musculus depressor mandibulae* complex), and 5) opener of the upper bill (protractor complex of the quadrate). The lines of action of the different muscles are given in figures 6.2. For each of these complexes the

Table 6.3. Maximal forces (N) on the bill tip as a result of muscle action. Values are given for elevation force of the upper bill, depression force of the upper bill, elevation force of the lower bill, and the force for simultaneous closing of the upper and lower bill. Column Max. elev. indicate the maximal elevation angle that can be obtained with these forces. >> indicate no limitation to rotation within the boundaries of the measured opposing forces.

Species	**Elev. UB (N)**	**Max. Elev. UB**	**Depr. UB (N)**	**Lower bill (N)**	**Max. Force (N)**
Struthio	11.7	56	61.0	24.4	24.6
Dromaius	15.0	>>	19.5	6.2	8.2
Rhea	11.2	38	39.3	12.6	14.6

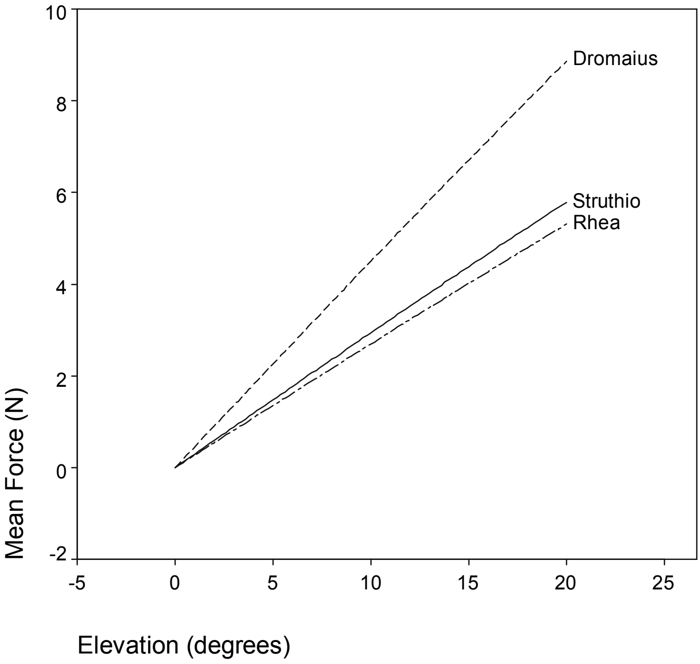


Figure 6.3. Reaction force in the upper bill versus the elevation angle of the upper bill. Forces were measured in the linear phase.

weights, fibre-lengths and maximal forces are given in table 6.1. The adductor complex consisted of two parts with different fibre lengths but a similar line of action. The forces of these two parts were combined for the final analysis.

Forces in the skull

The forces at the bill tip necessary to elevate the upper bill are relatively small (Fig. 6.3). Forces of 3 N will result in an elevation of the upper bill of more than 10 degrees. From the graph it is clear that the upper bill of *Dromaius* resists bending more than in the other two species. *Rhea* and *Struthio* show similar resistance to bending. Within the elevation range measured (0-15 degrees) the forces increase almost linearly.

The parameters of the four-bar systems are given in table 6.2. The main muscle responsible for the elevation of the upper bill is the *Musculus protractor pterygoideus et quadrati*. Depression of the upper bill from an elevated situation is achieved by the action of the *Musculus pterygoideus* and the adductor muscles attached to quadrate (Bühler, 1981). For the calculation of the forces on the bill tips of the subsystems the bills were considered to be in their rest position. The calculated forces on the bill tip as a result of the contraction of the *Musculus protractor pterygoideus et quadrati*, the simultaneously contraction of the *Musculus* *pterygoideus* and the adductors on the quadrate, and finally the *Musculus adductor externus* are given in table 6.3.

The forces on the bill tip as a result of the protraction of *Musculus protractor pterygoideus et quadrati* are comparable between species. The muscle forces and shape of the four-bar system are similar which indicates that all mechanisms are equally able to transfer the force of this muscle onto the bill tip. As can be seen in figure 6.5 this muscle force is sufficient for an elevation of the upper bill of approximately 30 degrees for all species (ignoring the force-length relationships of the muscle and the small change in direction of the muscles as the quadrate

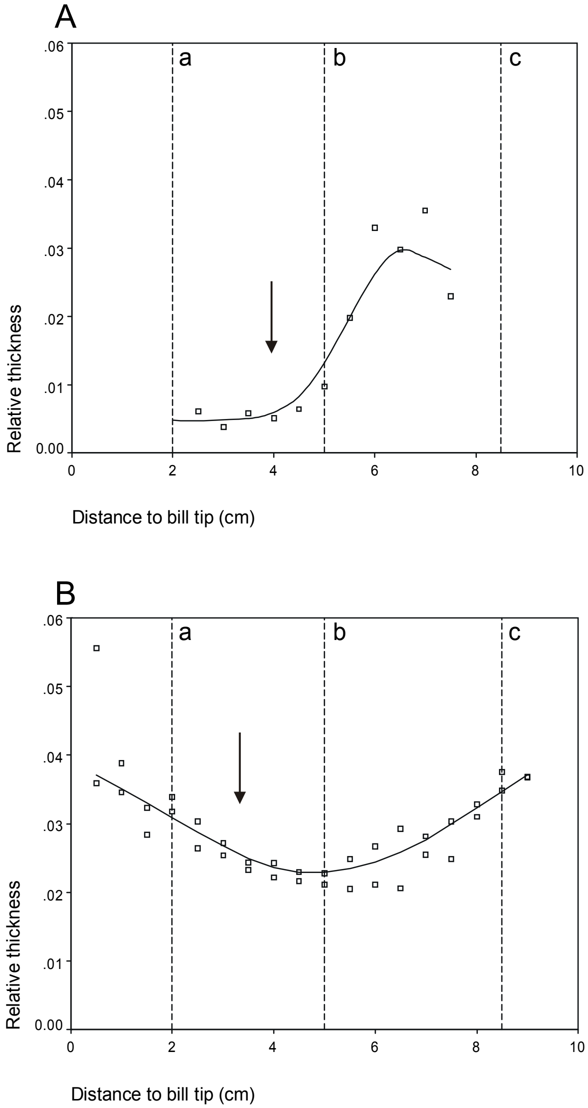


Figure 6.4. Bending zones in the upper bill elements of the Rhea (Rhea americana). Relative thickness of the dorsal bar (A) and ventral bar (B) vs. the distance from the bill tip. Lines are Lowess fits indicating average relative thickness. Vertical dashed lines indicate morphological characters of the bill: a) caudal border of the Rostrum maxillae, b) rostral point of the Rostrum basisphenoideus, c) position of the lateral bar. Arrows indicate the position of the bending zones as observed during experimental manipulation of the upper bill.

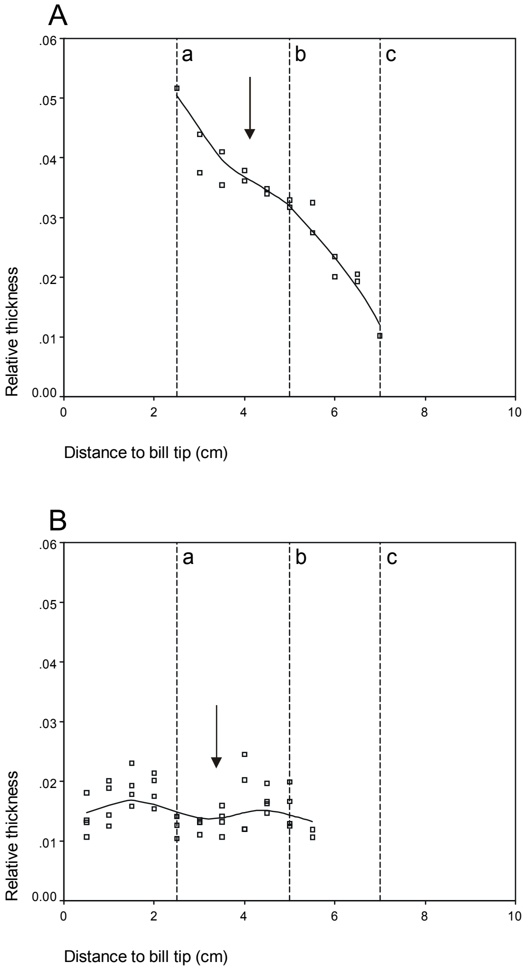


Figure 6.5. Bending zones in the upper bill elements of the Emu (Dromaius novaehollandiae). See figure 6.4 for details.

swings forward). The muscle forces resulting in a depression of the upper bill are larger than the elevation forces for all species. However, depression of the upper bill from rest position is prevented by the presence of the *Processus basipterygoideus*. Note that the large pterygoid muscles are also able to oppose external forces that would result in the elevation of the upper bill. Such external forces might act on the upper bill during grazing or when leaves are pulled from plants by a fast backward movement of the head and neck.

Large differences are found in the forces on the bill tip as a result of the closing muscles of the upper bill. The Ostrich is capable of producing a much larger force than the other two species. Since there are no large differences in orientation of the muscles, this is the effect of the higher mass of both the pterygoid and the adductor quadratum muscles in the Ostrich. The difference between the Rhea and the Emu can also be explained by the difference in muscle mass.

The differences in the forces on the bill tip produced by the *Musculus adductor mandibulae externus* are the result of differences in muscle mass, position of the muscles on the lower bill and the length of the bill. The high muscle mass of the Rhea does not result in the highest force output on the bill tip because the muscle attaches relatively close to the point of rotation in combination with a relatively long bill. The Ostrich has relatively the shortest bill, and therefore the most efficient force transfer. All forces on the bill tip produced by the *Musculus adductor externus* complex are however relatively small compared to those produced by the closing muscles of the upper bill.

The model including all head elements (Bout, *in prep.*) also showed that elevation of the upper bill had very little effect on the biting force. The force produced by the *Musculus* *pterygoideus* had also very little effect on the total biting force. Finally the contribution to the biting force of the *Musculus adductor mandibulae externus* is approximately twice as high as the contribution of the adductor muscles on the quadrate.

Bending Zones

When we consider the thickness of the ventral and dorsal bars of the upper bill of the different palaeognathous species (Fig. 6.4 - 6.6), it is clear that the thickness of the dorsal bar is related to the measured bending forces. The Emu with the highest resisting forces to elevation (Fig. 6.3) has a very thick dorsal bar and a very stable overall configuration (Fig. 6.5), while the Ostrich and Rhea have thinner bars (Figs. 6.4 & 6.6), which require lower bending forces (Fig. 6.3). In rhynchokinetic birds with known functional bending zones, such as *Calidris*, a clear bending zone can be recognised from the relative thickness of the bones (Fig. 6.7 & 6.8). These bending zones, characterised by a reduction of the thickness of the bones, coincide accurately with the position of the bending zones as determined from behavioural data. In Palaeognathae such zones of reduced thickness were not found, indicating that no clear bending zones are present in the upper bills of this taxon. Remarkable are also the differences in the morphology of the dorsal and ventral bars in the Palaeognathae. While in the *Calidris* species the dorsal bar follows the same pattern as the ventral bar with a clear bending zone, such a bending zone in the dorsal bar is absent in the Palaeognathae. Thinner regions can be found in the ventral bar, but these do not represent bending zones. In *Struthio* and *Rhea* the thinnest part in the dorsal bar is located directly caudal to the *Rostrum maxillae* and is relatively small. This position does not coincide with the bending zones found in kinematic analyses (Chapter 5 & 7) nor with the location suggested by other

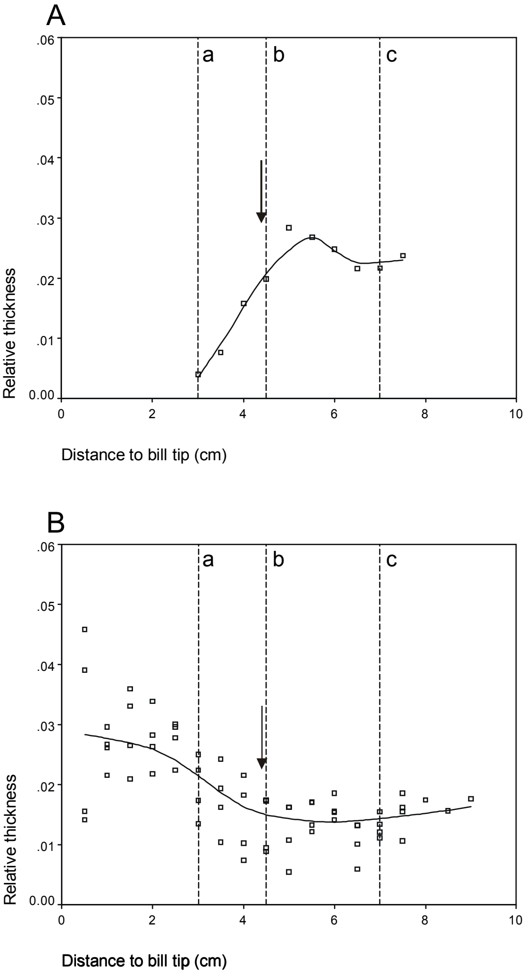


Figure 6.6. Bending zones in the upper bill elements of the Ostrich (Struthio camelus). See figure 6.4 for details.

authors (Hofer, 1954; Simonetta, 1960; Bock, 1963) In *Dromaius* the dorsal bar is relatively thick near the *Rostrum maxillae* and becomes thinner to caudal. This suggests that the bar would bend far more caudally than is expected from kinematic analyses. In fact bending at this point is impossible due to the presence of a thick *Rostrum parasphenoidale*.

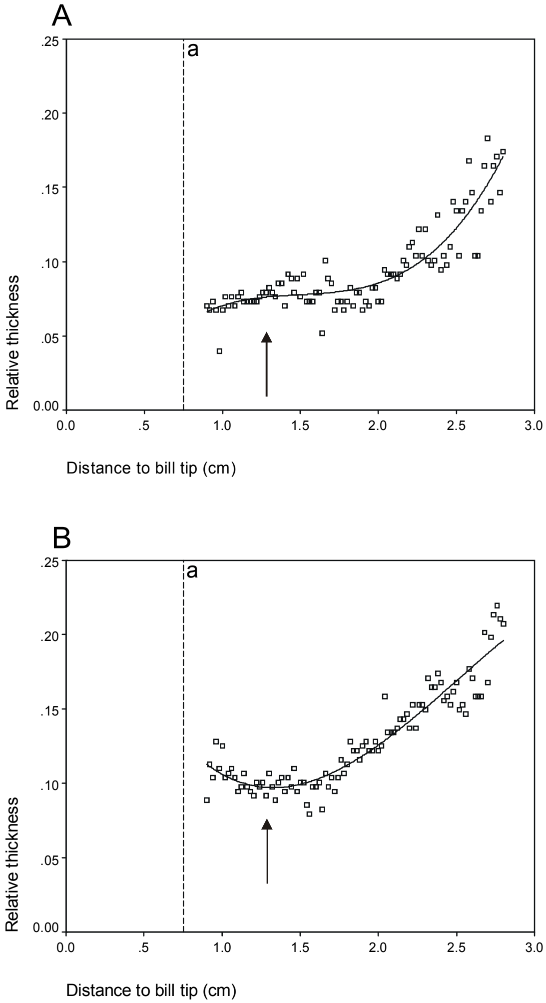


Figure 6.7. Bending zones in the upper bill elements of the Purple sandpiper (Calidris maratima). See figure 6.4 for details.

Discussion

A number of adaptations in the morphology of the skull of Palaeognathae are expected if they would have a fully functional form of rhynchokinesis. These adaptations have been described for other types of rhynchokinesis in neognathous birds (Zusi, 1984; Chapter 3). These adaptations first of all include the uncoupling of the dorsal and ventral bar of the upper bill, and the presence of bending zones in these elements. The uncoupling of the dorsal and ventral bar in neognathous rhynchokinetic birds is achieved through the schizorhinal nostril, which is not present in palaeognathous birds. The lateral bar in prokinetic birds is a rigid bony bar and in neognathous rhynchokinetic birds a flexible, but still bony, bar. In Palaeognathous birds this bar is reduced to two small bony protrusions from the ventral and dorsal bar connected by a ligament. This condition has probably not evolved from the morphology in neognathous rhynchokinetic birds, since the overall configuration of the palaeognathous nostril strongly resembles that of prokinetic birds (Zusi, 1984).

The second adaptation, the presence of bending zones in the upper bill is less clear in the Palaeognathae. In *Calidris* species, neognathous rhynchokinetic birds, bending zones are clearly recognisable in both the dorsal and ventral bar. The position of these bending zones coincides with the bending point found in behavioural analyses (Gerritsen, 1988). In the dorsal bars of the bills of Palaeognathae no slender areas, indicating bending zones, can be found, although the bars are relatively more slender than in *Calidris*. All the Palaeognathous species analysed in this study have the thinnest zones in the dorsal bar near the caudal or rostral part of the dorsal bar, outside the effective bending limits of the upper bill. This indicates that no clear bending zone can be found in the morphology of the Palaeognathae. Although the thickness-curve of the Ostrich shows some resemblance to that of the *Calidris* species it has to be noted that the slender part is directly behind the *Rostrum maxillae*. This *Rostrum* is very thick, rigid and semicircular, which makes bending in this region very unlikely. A behavioural analysis of the feeding of the Rhea has shown that limited bending in the upper bill occurs over the full length of the upper bill during feeding (Chapter 7). The ventral bar of the Palaeognathae shows a slight decrease in relative thickness, but this is far less pronounced than in the *Calidris* species. The thinnest parts of the ventral bars are positioned very caudally in the bill, far beyond the most rostral part of the *Rostrum parasphenoidale*. Since no bending can occur in the rigid *Rostrum parasphenoidale* it is very unlikely that bending does occur along this part in the ventral bar.

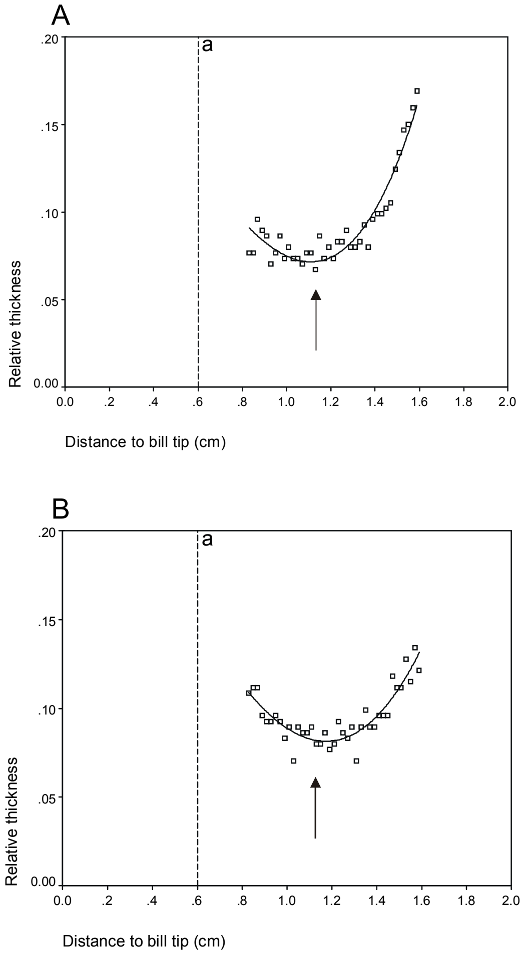


Figure 6.8. Bending zones in the upper bill elements of the Sanderling (Calidris alba). See figure 6.4 for details.

Another condition for a rhynchokinetic skull is the ability to move the upper bill and to overcome the reaction forces from bending. As is shown here, the bending forces in the upper bill of the Palaeognathae are relatively small (max 0.5 N/degree elevation). The available muscle forces are large enough to overcome the forces opposing elevation. The lack of clear bending zones indicates that the morphology of the Palaeognathae is not ‘designed’ for extreme elevation of the upper bill and agrees with earlier findings that the bill bends slightly, but over its full length. Due to the large *Musculus pterygoideus* complex, the forces that can be used to resist elevation of the upper bill by external forces are larger (Table 6.3). From a functional point of view these findings contradict the idea that the palaeognathous skull is rhynchokinetic.

It has generally been accepted that the palaeognathous PPC is related to rhynchokinetic feeding behaviour (Bock, 1963; Hofer, 1954; Simonetta, 1960). Our findings, however, show no evidence for this hypothesis. In the force analysis we found that the pterygoid muscles have a large component acting on the upper bill, which is stabilised by the presence of the *Processus basipterygoideus*. The strong *Musculus pterygoideus* can pull the PPC against the *Processus basipterygoideus*. By doing so, it is possible to resist strong external forces that might elevate the upper bill. The idea that the large pterygoid muscle in combination with the *Processus basipterygoideus* acts as a stabilising mechanism is also confirmed by the overall configuration of the skull. A large *Rostrum parasphenoidale* is found in combination with an almost completely ossified palate, with very broad bones. In neognathous birds the bones tend to be more slender, especially when no muscles are attached to it. The overall configuration seems to reflect a demand for stabilising of the upper bill and to enlarge the amount of bone in the palatal region.

Our findings also contradict part of the hypothesis of Zweers *et al.* (1997). They assume that the presence of a moveable palate in combination with a large pterygoid muscle results in higher biting forces. In our analysis we found that the *Musculus pterygoideus* has very little effect on the biting force and that elevation of the upper bill also has a very small contribution to the biting force. The function of the *Musculus pterygoideus* in birds that lack a blocking *Processus basipterygoideus* is to balance the retraction component of the adductor muscle, which tends to retract the lower bill and quadrate. In the Palaeognathae this retraction component is balanced by the *Processus basipterygoideus*. It is therefore very unlikely that additional biting force has resulted in the special palaeognathous configuration of the PPC.

Two different evolutionary pathways can be postulated to explain the morphology of the skull of the Palaeognathae. The first is based on the large increase in size of the species of the Palaeognathae. The ancestor of the Palaeognathae was a small species with an akinetic skull. With the increase in size of the birds the total weight of the skull decreased to reduce the weight on top of the neck. A reduction in bony elements already occurred during the transition from the ancestral reptilian skull to the present avian skull. The main reduction occurred in the lateral bars, which disappeared (Zweers *et al*., 1997). When the Palaeognathae, after reduction of the lateral bars, started to increase in size the skull needed an alternative method of reinforcement of the upper bill to resist unwanted bending due to external forces.

Prevention of large bending may be necessary to protect the upper bill itself and to ensure sufficient biting force, but may also prevent damage to other structures such as the olfactory organs. These are well developed in the Palaeognathae but have never been noticed as a significant feature. Within the large nasal cavity well-developed lamina are present, supporting the olfactory epithelium. In all Palaeognathae, including the Tinamidae, these cartilageous, or sometimes even bony, lamina are found and at least the Kiwi is known to have a well-developed sense of smell. It is possible that the morphology of the palaeognathous skull has evolved as a lightweight protective measure for the nasal cavity. When large bending occurs in the upper bill, the lamina in the nasal cavity may be damaged. The damage from this movement can be avoided by a stabilising the upper bill or by reducing the local displacement. The stabilising basipterygoid process that opposes depression of the upper bill, the strong pterygoid muscles that opposes elevation of the upper bill, and the absence of bending zones, protect the nasal cavity from movement damage. The evolutionary pathway in this case has either followed the same route of reduction of lateral bars as mentioned above, or the ancestral type had a rhynchokinetic skull. It has to be pointed out, however, that in both cases the bifurcation of the palaeognathous birds and the neognathous birds must have occurred early in evolution, since the long and broad vomer is still present. A reappearance of a long vomer from a situation as found in the neognathous birds where it is almost missing would be highly unlikely. We therefore have to conclude that the cranial characters of the Palaeognathae have evolved early in avian evolution and are not the direct result of a specific type of cranial kinesis.

Acknowledgements

We like to thank Yvonne de Man and Helen Jongeneel for the determination of maximal muscle forces and Merijn de Bakker for the staining of the bill of the Ostrich.

CHAPTER 7

EVOLUTIONARY IMPLICATIONS OF FEEDING BEHAVIOUR OF PALAEOGNATHOUS BIRDS

Summary

Cranial kinesis is an important feature in avian feeding behaviour and involves the transmission of quadrate movement to the upper bill by the Pterygoid-Palatinum Complex (PPC). The PPC in Palaeognathae is remarkably different from that found in Neognathae. In this study we analyse whether the special morphology of the PPC is related to the feeding behaviour of the Rhea (Rhea americana). The feeding behaviour of the Rhea is typical 'Catch & Throw' behaviour independent of the size of the food-item. Drinking is achieved by a scooping movement followed by a low-amplitude tip-up phase. Neither the feeding nor the drinking behaviour require adaptations different from those found in Neognathous birds, which indicates that the specific morphology of the PPC is not the result of specific functional demands from palaeognathous feeding behaviour. Cranial kinesis is limited and observed during gaping only. The feeding behaviour of the Palaeognathae is either derived optimised 'Catch & Throw' behaviour or primitive within birds.

Introduction

Since Merrem (1813) separated the Palaeognathae from all other birds, the taxon Palaeognathae has caused many disputes among ornithologists. One of the most important characters that separates the Palaeognathae from all other modern birds (Neognathae) is the dromaeognathous (= palaeognathous) palate, first described by Huxley (1867). Not only the ‘palate’ is different from that found in Neognathae but a whole complex of morphological characters, which includes structures such as the pterygoid, quadrate and vomer (see McDowell, 1948; Bock, 1963; Gussekloo & Zweers, 1999). The set of characters of the jaw mechanism that discriminates the Palaeognathae from the Neognathae will be referred to as the Palaeognathous Pterygoid-Palate Complex (Palaeognathous PPC; Gussekloo & Zweers, 1999). The mechanical function of this Pterygoid-Palate Complex in neognathous birds is well known. In these birds the PPC participates in the movement of the upper bill (Bock, 1964). Upper bill movement is induced by rostrad rotation of the quadrate, which pushes both the lateral jugal bars and the medial pterygoid-palate bar forward. Each bar transfers its forces and movement onto the premaxilla. The forward movement of the premaxilla results in an upward rotation of the upper bill around a hinge, either in the nasal-frontal area (prokinesis) or in the rostral part of the bill (rhynchokinesis), depending on the position of the flexible zone. The pterygoid-palate bar and the quadrate are of great importance, since the muscles for the movement of the upper bill attach to these elements. Although many authors have used the palaeognathous PPC for systematic purposes (Fürbringer, 1888; Gadow, 1892; Beddard, 1898; McDowell, 1948; de Beer, 1956; Bock, 1963), the number of authors that has studied the function of the system is very limited. In all functional analyses it was assumed that the special morphology of the PPC in Palaeognathae is related to rhynchokinesis (Hofer, 1954; Simonetta, 1960; Bock, 1963). This assumption was based on the osteology of the PPC, the flexibility of the dorsal and ventral bars of the upper bill, and the incomplete ossification of the lateral bar (Zusi, 1984). The occurrence of rhynchokinesis has, however, never been tested in alive birds.

Several hypotheses can be postulated about the evolution of the special PPC morphology in the Palaeognathae. First, different selective forces may have acted directly on the PPC and upper beak movement of the Neognathae and of the Palaeognathae, resulting in a difference in morphology. These selective forces might be the direct effect of differences in the role of the PPC. In this analysis the function of the PPC is considered to be the transfer of forces from the quadrate onto the upper bill, and its role the elevation or depression of the upper bill. To investigate whether differences in selection forces on bill elevation, and therefore on the PPC, are present, upper bill movement of the Palaeognathae during feeding will be described and compared with its function and role in a general neognathous-feeding pattern. If no differences can be found in the present function of the PPC between Neognathae and Palaeognathae it is plausible that no differences in selective forces are present.

Second, when differences in selective forces are not responsible for the difference in morphology other mechanisms must have played a role. Alternative explanations would be that the specific morphology of the palaeognathous PPC is an epi-phenomenon or is the result of evolution under the same selective forces but from a different starting design or within different developmental constraints. The historical explanation (different starting design) is complicated because it is not yet known whether the Palaeognathous configuration of the PPC represents a primitive or a derived condition within the phylogeny of modern birds.

In this study we investigated whether the morphology of the PPC in Palaeognathae is an adaptation to its present feeding behaviour. Feeding behaviour is considered the strongest selection force acting on the upper bill opening mechanism and the PPC is one of the key-elements within that mechanism. Other behaviours such as vocalisation, preening and social behaviour are considered to have little effect on the osteology of the bills. Once the feeding behaviour and especially the use of upper bill kinesis of the Palaeognathae is known, it is possible to compare it with the general neognathous feeding pattern. This comparison between the two feeding behaviours might indicate differences in selective forces acting on the PPC in Neognathae and Palaeognathae and explain the differences in the morphology of the PPC. For this analysis feeding will include only the behavioural elements from picking-up the food-item until swallowing. All phases prior to the picking-up for intra-oral transport are considered a part of food-acquisition.

For a functional-evolutionary analysis of feeding behaviour of an animal it is necessary to know the natural food-types of the animal, and to determine the general feeding behaviour and the flexibility of the general pattern. When these are known, functional demands for the morphology can be postulated, and it can be determined whether the morphology is an

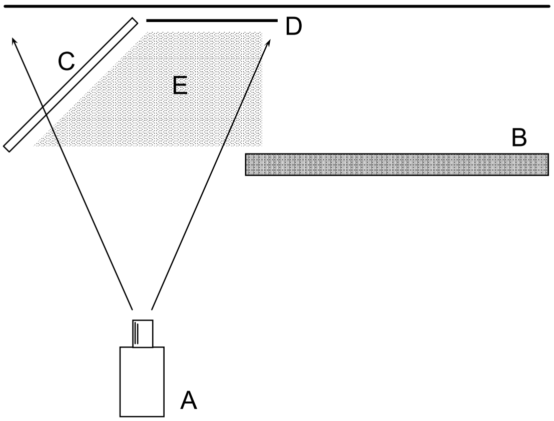


Figure 7.1. Experimental Set-up in top view. The Video camera (A) captures an image of the feeding arena (E), a reference grid (D, squares 2x2 cm), and an indirect frontal image of the animal via a mirror (C). A corridor (B) is used to force the birds into a lateral position relative to the camera at the feeding arena.

adaptation to the specific functional demands of palaeognathous feeding. In this study a comparison is made between the feeding behaviour of the Palaeognathae and the general neognathous feeding patterns as described by Zweers *et al.* (1994). Kinematic differences are identified that might indicate differences in selective forces on the PPC. Furthermore, both morphology and behaviour can be used for outgroup comparison to determine whether the palaeognathous PPC configuration represents a primitive or derived condition.

Table 7.1. Markers on the head of the Rhea, markers are also shown in figure 7.2.

No.	**Marker**
1	Rostral edge of the eye
2	Caudal edge of the eye
3	Centre of the ear
4	Centre of the food item
5	Upper Bill, near the bill tip (ventral edge)
6	Upper Bill, rostral of the bending zone (ventral edge)
7	Upper Bill, caudal of the bending zone (ventral edge)
8	Upper Bill, caudal part (ventral edge)
9	Upper Bill, most rostral point with feathers
10	Lower Bill, near the bill tip (dorsal edge)
11	Lower Bill, rostral of the bending zone (dorsal edge)
12	Lower Bill, caudal of the bending zone (dorsal edge)
13	Lower Bill, caudal part (dorsal edge)
14	Throat, near end rhamphotheca
15	Throat, near end lower jaw
16	Throat, two centimetres below marker 15

Materials and Methods

The Greater Rhea (*Rhea americana*), a middle-sized palaeognathous bird from South-America, was chosen as representative for the Palaeognathae. This Rhea has a general palaeognathous PPC configuration (McDowell, 1948) and its natural history and behaviour are well known (Raikow, 1968; Raikow, 1969; Bruning, 1974; Martella *et al.*, 1995; Martella *et al.*, 1996; Reboreda & Fernandez, 1997). For the analysis two specimens, one male and one female, were trained to feed on several food-types within the experimental set-up. The feeding behaviour of the birds was recorded using video imaging (25 frames per second). The recordings were made in an experimental set-up in which a lateral view and a frontal view of the bird were obtained in the same frame using a mirror situated in front the bird at an angle of 45 degrees (Fig. 7.1). The birds had to approach the feeding arena through a small corridor ensuring a good lateral position of the bird with respect to the camera. Behind the bird, from the

Table 7.2. Calculated parameters used in the kinematic analysis.

No.	Description	Measure
1	Standard measure, measured on the reference grid	Millimetres
2	Gape, distance between upper and lower bill tip	Millimetres
3	Distance food, distance between the food item and the upper bill tip	Millimetres
4	X Position Head, relative horizontal position of the head (ear)	Millimetres
5	Y Position Head, elevation of the head (ear) above the ground	Millimetres
6	Flexion in nasal-frontal hinge, angle between cranium and caudal part of the upper bill	Degrees
7	Flexion halfway the upper bill, angle between the caudal and rostral part of the cranium	Degrees
8	Opening lower bill, angle between the cranium and lower bill	Degrees
9	Depression of the throat, distance between the cranium (ear) and oropharynx floor near the larynx.	Millimetres

camera’s point of view, a grid (squares 2 x 2 cm) was placed to make scaling possible. The films were analysed, frame-by-frame, by digitising the position of several points on the upper and lower bill relative to the standard grid (Fig. 7.2, Table 7.1). In addition to these points on the bills, some reference points on the skull of the bird were also digitised (Fig. 7.2, Table 7.1). From the complete set of digitised points a number of distances and angles was calculated, which are summarised in table 7.2. The accuracy of the calculated distances and angles were determined on the basis of the variation in a standard measurement calculated as the distance between two digitised points of the reference grid. The error in digitising a point was approximately 0.4 cm, the error in distance measures approximately 0.6 cm. The errors for points were used to calculate the error for angles. For angles the error is dependent on the distance between the points and the angle between lines. We estimated the error for the upper bill rotation to be 7 degrees for angles of 10 degrees. The markers used for the calculation of angles were approximately in the range of these errors.

The data on head displacement were used to determine maximum velocities and accelerations of the head during feeding. The complete movement of the head was determined by interpolation to 250 points per second using a cubic spline interpolation technique. The spline interpolation technique was used under the assumption that head movements follow a gradual and symmetric path around the points of change of direction. Behavioural observations confirm these assumptions. The interpolated data were used to calculate both velocities and accelerations. The acceleration data in combination with the weight of the head (estimated from the weights of heads in other individuals) were used to determine the forces acting on the head.

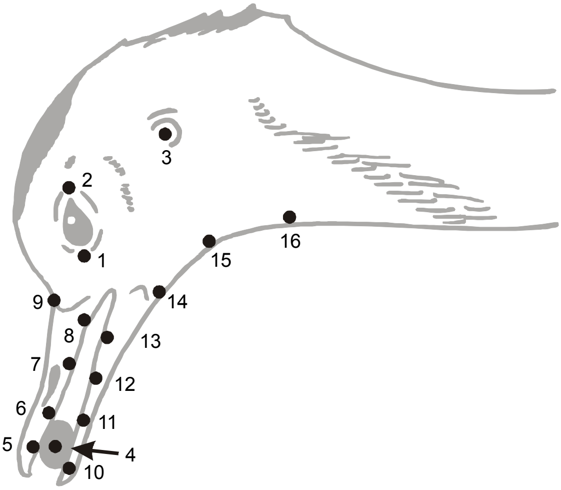


Figure 7.2. Digitised points in each frame of the feeding scenes. Numbers refer to table 7.1. In addition to the points shown three standard points on the background grid were digitised to determine horizontal and vertical axis and to scale the images.

A range of food-types was offered (Table 7.3). The size of the different food-types varied between 4 mm and 35 mm in length and all food-types were offered individually. For the analysis of each bird, at least five items of each food-type were analysed. Large apples were only eaten by the male and only three times. Drinking cycles were observed in both individuals, but only seven cycles could be analysed. Only separate food-items were offered since our preliminary observations showed that the transport of individual food-items is very similar to the transport during grazing, when leaves are removed from the plant by sudden head jerks and transported into the oesophagus. The effect of this type of food-acquisition will be discussed later.

To investigate the diversity and variability of the feeding behaviour, a Principle Component Analysis was used to describe the variation in feeding behaviour due to different food-types. The PCA, with Varimax rotation, was based on the correlation matrix of characters. The characters were obtained from the movement patterns of the different head elements important in feeding (Table 7.4, Figs. 7.5 & 7.6). Differences in Principal Component scores were determined with an Analysis of Variance (ANOVA).

The natural food preferences of the Greater Rhea were obtained from the work of Martella *et al.* (1996). Prior to the feeding analysis the position of the bending zones was determined through manipulating osteological specimens. The found positions were compared to previous descriptions (Hofer, 1954; Simonetta, 1960; Bock, 1963; Zusi, 1984) and used to determine the position of points for digitising.

Results

1. General feeding and drinking behaviour

A general feeding sequence of the Rhea (Fig. 7.4a & 7.5) consists of the following elements: the bird approaches the food-item while opening the bills. A fixation phase as in the general model for neognathous feeding described by Zweers (1994) could not be distinguished. The food-item was picked-up, sometimes followed by repositioning behaviour. Repositioning occurred more often when large food-items were eaten than when small food-items were eaten. Although it has been suggested that the special morphology of the PPC might be an adaptation to the high impact forces on the bill during pecking (Bock, 1963), the movement analysis showed that the Rhea is capable of limiting the impact force of pecking. When the head hit the ground the acceleration of the head was approximately 11.30 m/s^2 ($a = 11.30 \pm 6.57$ m/s^2, n=41). With an estimated weight of the head of 0.25 kg, the maximum calculated impact force did not exceed 7.54 Newton ($a_{max} = 30.17$ m/s^2).

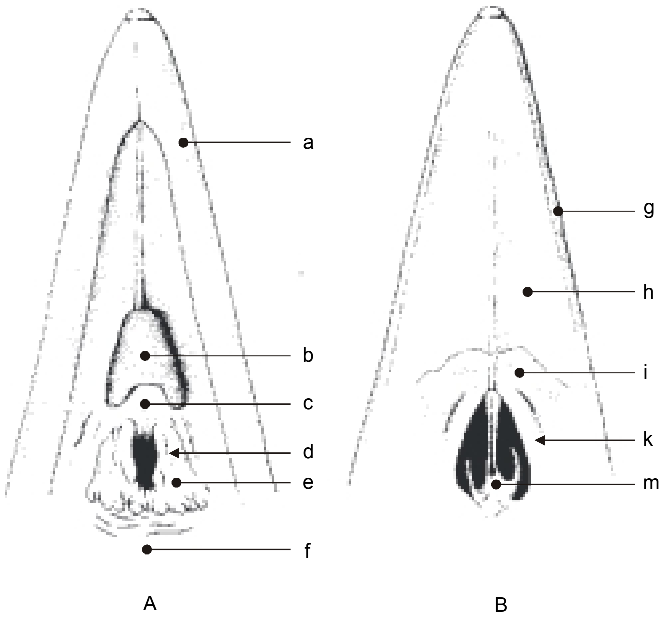


Figure 7.3. Oropharynx of the Greater Rhea (Rhea americana). A. dorsal view of oropharynx floor. B ventral view of oropharynx roof. a) rhamphotheca, b) tongue cushion, c) tongue base, d) opening of the trachea, e) larynx, f) esophagus, g) rhamphotheca, h) bony palate, i) soft palate, k) choana, m) vomer.

When the food-item was correctly positioned a single 'Catch & Throw' movement was used to transport the food-particle into, or near to, the entrance of the oesophagus. A 'Catch & Throw' movement starts when the food is fixed between the bills, the head is accelerated upward and slightly backward. Then the bills open and the head is suddenly moved forward. The accelerated food-item continues to move upward while the head of the bird moves downward, which results in the transport of the

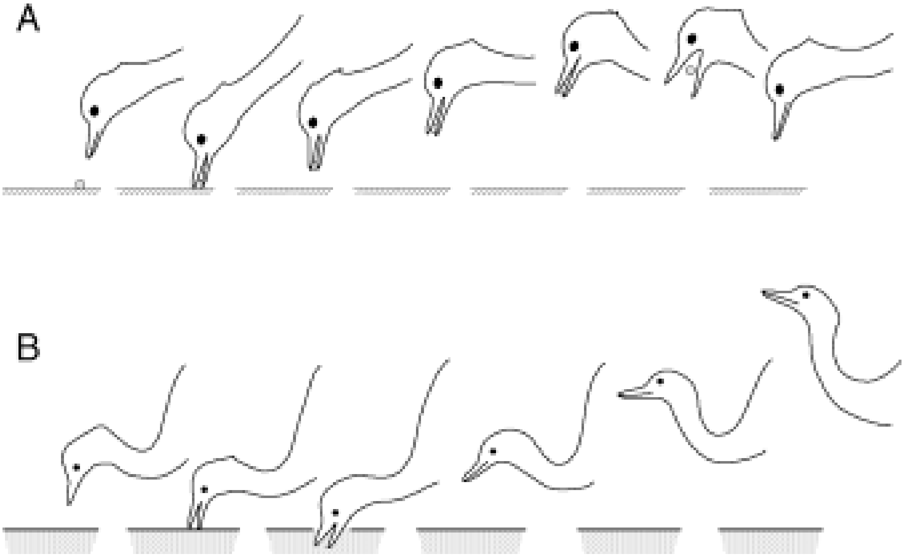


Figure 7.4. Outline drawings of feeding behaviour (A) and scoop drinking behaviour (B) of the Greater Rhea (Rhea americana). Horizontal lines represent ground level (A) or water level (B).

food-item. During the ‘Catch & Throw’ movement the floor of the mouth and pharynx were pulled downward which increases the total volume of the buccal cavity. No tongue movement was observed other than the one resulting in the depression of the mouth floor.

The Rhea used two different types of drinking behaviour, depending on the area of water available to drink from. The preferred method of drinking can be described as scoop drinking followed by a low-amplitude tip-up phase (Fig. 7.4b). In this behaviour the bird opens the bill, inserts it into the water, and with a forward scooping motion of the head the bill is filled. The bill is then closed and the head is elevated until the neck is almost completely stretched, while the head itself is in a horizontal position. Finally, the water is transported into the oesophagus by a slight elevation of the bill tips and a retraction of the tongue. In some cases a small horizontal ‘Catch & Throw’ movement may occur just prior to swallowing. When the size of the water surface limits the

Table 7.3. Approximate dimensions of offered food types

No.	Food type	Dimensions (mm)
1	Apple large	35 x 35 x 35
2	Apple small	25 x 25 x 25
3	Pellets	10 x 10 x 25
4	Seeds	4 x 3 x 8
5	Water (drinking)	-

scooping movement, the Rhea used a drinking technique that is very similar to pecking behaviour. The bill is opened and inserted almost vertically into the water, the bill is then closed and in a single head jerk the water is accelerated vertically, the bill is opened and the water is transported to the back of the oropharynx. Since this behaviour strongly resembles pecking, and is not the basic drinking behaviour it was not included in this analysis.

Although feeding behaviour was analysed under controlled conditions, field data show that the observed feeding behaviours are present in the natural behaviour of the Rhea as well. The

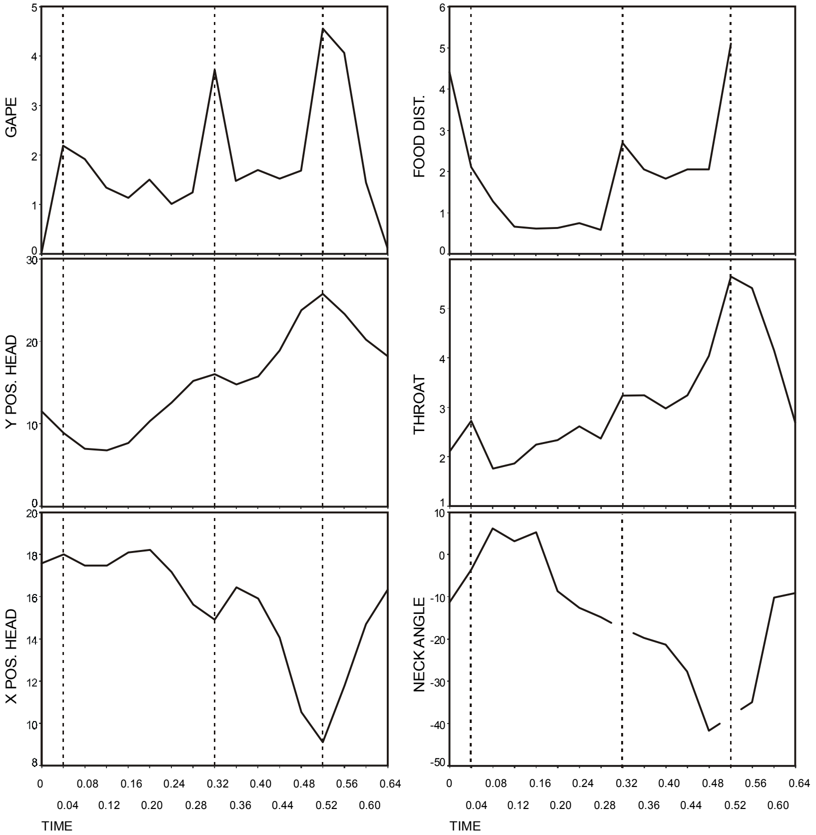


Figure 7.5. A characteristic feeding cycle of the greater rhea (Rhea americana). The vertical dashed lines indicate characteristic moments in the feeding cycle. In chronological order: picking up the food item (grasp), maximum gape during a repositioning cycle and maximum gape during the transport phase. The graphs show the gape, the vertical and horizontal position of the head, the distance between the food item and the bill tips, the depression of the throat and the flexion of the most rostral part of the neck.

single ‘Catch & Throw’ feeding behaviour and both the scooping and ‘Catch & Throw’ drinking behaviour have been observed by Greater Rhea in the wild (Navarro, personal communication, 1998). We also observed the single ‘Catch & Throw’ feeding behaviour in wild and captive Ostriches (*Struthio camelus*) and captive Emus (*Dromaius novaehollandiae*) and cassowaries (*Casuarius casuarius*). These observations indicate that the drinking/feeding behaviour of the Rhea is characteristic for most Palaeognathae.

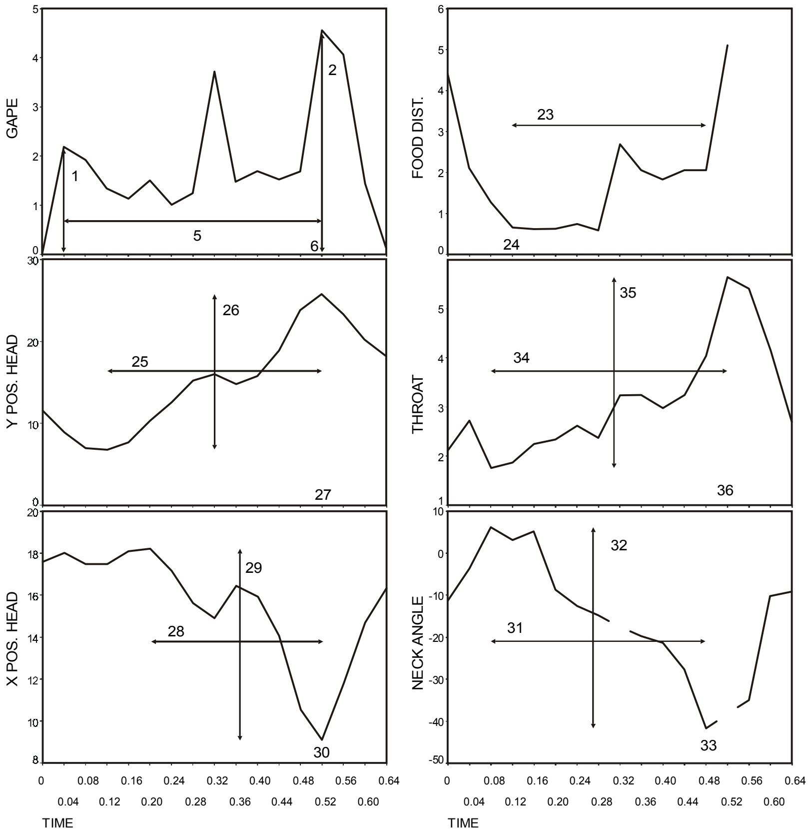


Figure 7.6. Measurements selected to describe the feeding behaviour of the greater rhea and used in the principal component analysis. Numbers in the graphs refer to table 3. Measurements from the groups 'gape', 'lower bill', 'prokinesis' and 'rhynchokinesis' are correlated to the maximum gape during food pecking (gape 1; no. 1) and the maximum gape in the transport phase (gape 2; no. 2). Graphs of prokinesis, rhynchokinesis and lower bill depression are not given but they strongly resemble the pattern shown in the gape graph.

Table 7.4. Measured Parameters

No.	Group	Parameter	Description
1	Gape	Gape 1	Maximum gape during the approach
2		Gape 2	Maximum gape during ‘Catch & Throw’
3		Gape Level	Mean gape between Gape 1 and 2
4		Gape Level Std	Standard deviation of measure 4(Indicator for repositioning)
5		Gape Period	Time between Gape 1 and 2
6		Gape 2 Moment	Time from start to Gape 2
7	Lower Bill	Lower Bill 1	Maximum depression of the lower bill during the approach
8		Lower Bill 2	Maximum depression of the lower bill during the ‘Catch & Throw’
9		Lower Bill Level	Mean depression of the lower bill between Lower bill 1 and 2
10		Lower Bill Std	Standard deviation of measure 9 (Indicator for repositioning)
11		Lower Bill Period	Time between Lower bill 1 and 2
12		Lower Bill 2 Moment	Time from start to Lower bill 2
13	Pro.	Prokinesis at Gape 1	Angle around nasal-frontal hinge at Gape 1
14		Prokinesis at Gape 2	Angle around nasal-frontal hinge at Gape 2
15		Prokinesis Level	Mean angle around nasal-frontal hinge between Gape 1 and Gape 2
16		Prokinesis Level Std	Standard deviation of measure 15 (Indicator for constancy of kinesis)
17	Rhyncho.	Rhynchokinesis at Gape 1	Angle around bending zone in the upper bill at Gape 1
18		Rhynchokinesis at Gape 2	Angle around bending zone in the upper bill at Gape 2
19		Rhynchokinesis Level	Mean angle around bending zone in the upper bill between the moment of Gape 1 and Gape 2
20		Rhynchokinesis Level Std	Standard deviation of measure 19 (Indicator for constancy of kinesis)
21	Food	Food Level	Mean distance between the cranium and the food-item between the moment of grasping and swallowing
22		Food Level Std	Standard deviation of measure 21(Indicator for inter-oral transport other than ‘Catch & Throw’)
23		Food Period	Duration of holding the food item
24		Food Min Moment	Moment of release of the food item in the ‘Catch & Throw’
25	Head Y	Head Elevation Period	Time between minimum and maximum elevation of the head
26		Difference Head Elevation	Maximum distance of head elevation
27		Max Head Elevation Moment	Moment of maximal head elevation
28	Head X	Head X Period	Time between minimum and maximum horizontal displacement of the head
29		Difference Head X	Maximum distance of horizontal head displacement
30		Min Head X Moment	Moment of minimal horizontal extension of the neck (head closest to the body)
31	Neck	Neck Neck Period	Time between maximal and minimal flexion of the neck
32		Difference Neck Neck	Difference in angle between maximal and minimal flexion
33		Neck Neck Moment	Moment of minimum neck flexion
34	Throat	Throat Period	Period between minimum and maximum throat depression
35		Difference Throat	Distance between minimum and maximum throat depression
36		Throat Moment	Moment of maximum throat depression

Table 7.5. Mean values per food type for all characters used in the Principle component analysis. Values are measurements relative to either the cranium or the reference grid.

No.	**Group**	**Parameter**		**Food 1 Apple large (n=3)**	**Food 2 Apple small (n=11)**	**Food 3 Pellets (n=12)**	**Food 4 Seeds (n=14)**	**Food 5 Water (n=7)**
1	Gape	Gape 1	cm	2.80	2.31	2.09	1.49	4.66
2		Gape 2	cm	7.53	5.47	5.24	2.43	0.00
3		Gape Level	cm	3.46	1.88	1.67	0.52	0.37
4		Gape Level Std	cm	0.74	0.86	0.72	0.55	0.90
5		Gape Period	frames	18.33	14.73	17.18	7.86	21.43
6		Gape 2 Moment	frames	17.67	13.82	15.73	6.36	19.71
7	Lower Bill	Lower Bill 1	degrees	32.37	42.94	41.23	41.16	40.20
8		Lower Bill 2	degrees	58.70	55.60	55.46	45.98	0.00
9		Lower Bill Level	degrees	39.06	38.50	39.74	32.74	25.56
10		Lower Bill Std	degrees	8.60	5.87	5.48	3.82	6.50
11		Lower Bill Period	frames	18.67	14.27	17.27	7.79	21.14
12		Lower Bill 2 Moment	frames	18.00	13.45	16.00	6.29	19.71
13	Prokinesis	Prokinesis at Gape 1	degrees	20.58	21.94	21.83	23.42	13.53
14		Prokinesis at Gape 2	degrees	18.60	21.49	23.44	22.68	22.36
15		Prokinesis Level	degrees	21.00	22.29	22.50	25.37	17.19
16		Prokinesis Level Std	degrees	4.11	3.98	3.28	3.66	5.04
17	Rhyncho.	Rhynchokinesis at Gape 1	degrees	6.12	6.19	8.92	4.29	-4.48
18		Rhynchokinesis at Gape 2	degrees	2.29	2.39	3.65	4.56	3.52
19		Rhynchokinesis Level	degrees	3.50	3.29	5.15	2.29	0.99
20		Rhynchokinesis Level Std	degrees	5.53	4.78	4.63	4.75	7.17
21	Food	Food Level	cm	1.91	1.43	1.46	1.33	3.37
22		Food Level Std	cm	0.62	0.60	0.66	1.47	1.88
23		Food Period	frames	15.33	12.45	15.36	6.64	23.71
24		Food Min Moment	frames	-1.00	-0.27	-0.58	-1.14	-0.29
25	Head Y	Head Elevation Period	frames	17.67	12.90	15.45	6.64	20.43
26		Difference Head elevation	cm	25.03	21.71	21.24	15.23	84.92
27		Max Head elevation Moment	frames	18.00	12.70	15.09	5.93	20.00
28	Head X	Head X Period	frames	12.33	12.73	10.45	6.64	8.43
29		Difference Head X	cm	3.92	9.37	8.27	5.86	6.13
30		Min Head X Moment	frames	16.00	11.18	15.09	5.36	7.86
31	Neck	Neck Neck Period	frames	16.00	12.45	15.55	5.69	5.43
32		Difference Neck Neck	degrees	46.81	57.11	50.99	32.82	36.26
33		Neck Neck Moment	frames	16.00	12.27	15.18	5.08	4.86
34	Throat	Throat Period	frames	17.33	14.91	17.54	5.79	12.00
35		Difference Throat	cm	5.09	3.09	3.41	2.51	2.36
36		Throat Moment	frames	17.67	14.09	15.91	6.64	6.86

2. Quantitative differences between food-types

To characterise the movement patterns quantitatively thirty-six parameters were chosen (Table 7.4 & 7.5, Fig. 7.5 & 7.6) and analysed using a Principal Component Analysis. The first three principal components of the PCA based on the characters of the feeding and drinking behaviours described 63% of the total variance. An analysis of variance (ANOVA) over the principle component scores was used to determine the main differences between individuals/sexes and food-types. None of the first three principle components showed a difference between individuals/sexes (df=47, PC1: F=0.264, p=N.S.; PC2: F=0.198, p=N.S.; PC3: F=0.240, p=N.S.) and therefore the data from both individuals were combined. It is clear from the plot of the first principle component (PC1) against the second principle component (PC2) that drinking behaviour is remarkably different from feeding behaviour (Fig. 7.7, Table 7.6). The first principal component describes the absence of the second gape movement ('Catch & Throw' movement), differences in neck movement (duration of the neck cycle) and the duration of the total feeding cycle (Table 7.7). The second principle component describes differences in food manipulation by the bills such as, position of the food-item between the bills, depression of the lower bill and kinesis. To investigate the differences between food-types without the effect of drinking, the PCA was repeated using the four types of feeding behaviour only. In this analysis 65% of the variance was explained by the first three principle components. To test whether there are significant differences between food-types a one-way analysis of variance over the first three principle components scores was used. Differences between the food-types were tested using a t-test with Bonferroni correction. There are large differences between food-types on both the first and third principal component (df=40, PC1: F=28.678, $p<0.001$; PC2: F=0.365, p=N.S.; PC3: F=3.628, $p<0.05$). It is clear that PC1 describes the effect of food size (Fig. 7.8). The change in movement along PC1 becomes smaller when the size of the food-items increases. A difference on PC1 is only found between the seeds and all other food-types (food-type 1 vs. 2, 3 and 4, t-test, Bonferroni correction, $p<0.001$) and on PC3 between the large apple and seeds (food-type 1 vs. 4, t-test, Bonferroni correction, $p<0.05$).

The differences on the first principle component represent mainly the effect of the duration of the movement for each food-type (e.g. Gape period, Head Elevation Period, Food Period, Lower Bill Period) the size of the first gape (Gape 1) and the elevation of the head (Difference Head Y; Table 7.7). All these parameters increase with an increase of the size of the food-type. This indicates that the movement pattern of food uptake is relatively constant and only the duration, mainly the effect of repositioning, is variable. The third principle component mainly describes the handling of the food-item, which affects the amount of depression of the lower bill (Lower bill at Gape 1), position of the food-item between the bills during the upward movement of the head (Food Level, Food Level Std) and amount of cranial kinesis (e.g. Prokinesis at Gape 1 &2, Rhynchokinesis at Gape 2, Table 7.7). Differences between food-types on PC3 are only found between large apples and seeds. However, no clear trends can be determined with a change in size of the food-types.

3. Cranial kinesis

To test the level of kinesis in the skull of the Rhea a number of measurements was taken. The movement between the cranium and the upper bill around the point were in prokinetic birds the nasal-frontal hinge would be, was measured and will be further referred to as prokinetic movement. A second measure was the movement between the rostral and caudal part of the upper bill with the border of the two parts in the bending region of the upper bill. This bending region was determined by manipulating two osteological specimens (skulls) and measuring the distance between the bill tip and the point of maximal bending. Movement of the rostral part of the upper bill will be referred to as the rhynchokinetic movement. Since food-types are different in size, the kinesis of the upper bill was determined for each food-type separately. The large apple was not used for this analysis due to the small number of repeated experiments.

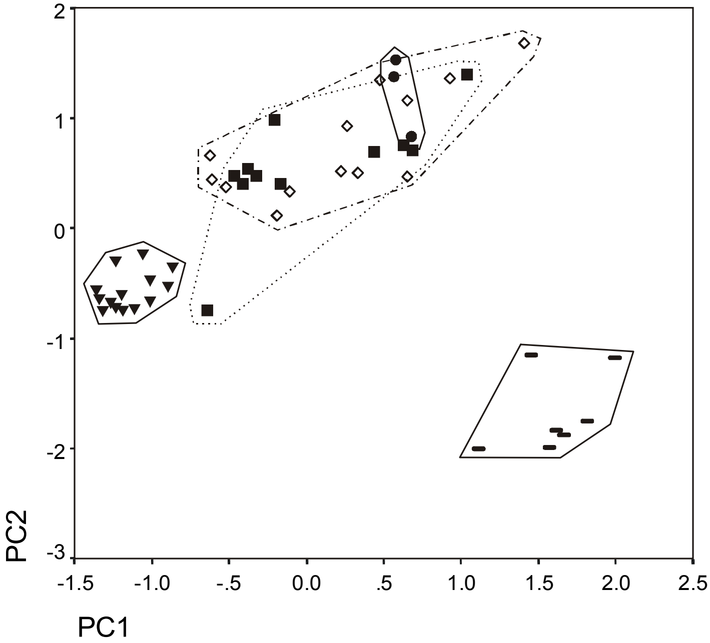


Figure 7.7. Plot of PC1 against PC2 of the analysis including drinking behaviour. Different food types are represented by different markers, and groups of a single food type are outlined. Circles: large apple, closed squares: small apple, open squares: pellets, triangles: seeds, single dashes: drinking behaviour.

It is assumed that maximal kinesis is observed during the large amplitude gapes when the food-item is picked-up or swallowed. Velocities of the head are very large during the second phase of the 'Catch & Throw' movement, which makes it very difficult to determine points accurately. Because of this low accuracy and the relatively small movements in the upper bill, cranial kinesis could be analysed only in the grasping phase. From repeated experiments the average pick-up cycle was calculated and plotted with the standard error. The standard error includes both measuring error and variation of the mean cycle. If time elements differ

Table 7.6. Main parameters contributing to the first three principle components of the total analysis. For PC1 only parameters with loadings higher than 0.8 are selected, for PC2 and PC3 parameters with loadings higher than 0.4 are selected. Percentages indicate the explained variance on each PC.

Character	**PC1 (45%)**	**PC2 (10%)**	**PC3 (9%)**
PC1			
Throat Moment	0.983	0.063	0.037
Gape 2 Moment	0.981	0.081	0.054
Lower Bill 2 Moment	0.977	0.038	0.087
Food 2 Moment	0.975	0.054	-0.012
Gape Period	0.974	0.082	0.043
Head Elevation Moment	0.966	0.091	0.093
Head Elevation Period	0.965	0.068	0.077
Lower Bill Period	0.956	0.119	0.103
Food Period	0.951	0.029	-0.029
Throat Period	0.946	0.008	-0.069
Neck Neck Moment	0.911	0.196	0.178
Neck Neck Period	0.907	0.183	0.191
Min Head X Moment	0.881	0.200	0.090
PC2			
Food Level	0.091	0.428	0.055
Gape Level Std	0.274	0.422	0.592
Food Level Std	-0.247	0.404	-0.047
Lower bill Level	0.634	-0.435	-0.002
Lower bill at Gape 1	0.103	-0.565	-0.385
Prokinesis Level	-0.381	-0.628	-0.162
Prokinesis at Gape 2	0.023	-0.683	0.173
Prokinesis at Gape 1	-0.175	-0.714	0.102
PC3			
Throat displacement	0.362	0.123	0.672
Difference Neck Neck	0.417	-0.263	0.504
Lower Bill Level Std	0.416	0.397	0.486
Lower Bill at Gape 2	0.571	-0.089	0.451
Difference Head Elevation	0.729	-0.064	0.443
Rhynchokinesis Level Std	-0.043	-0.075	0.438
Rhynchokinesis at Gape 1	0.348	0.026	-0.410
Rhynchokinesis at Gape 2	-0.038	0.332	-0.679

significantly from each other, the displacement in that period is larger than the measuring error and therefore the movement is significant.

The plot of gape versus time shows a clear pattern (Fig. 7.9) similar to a single food uptake cycle, and differences between the time segments are significant (ANOVA, food-type 2: df=85, $p<0.001$; food-type 3: df=90, $p<0.001$; food-type 4: df=168, $p<0.001$; food-type 5: $p<0.001$). This pattern can be found in angle-based calculations, which have a higher measurement error. A similar analysis was made for the lower bill movement expressed as the depression angle (Fig. 7.9). For all food-types the same pattern was found, and only for the largest analysed food-type (food-type 2: Small Apple) the differences between times were not significant due to large

Table 7.7. Main parameters contributing to the first three principle components of the food type analysis. For PC1 and PC2 only parameters with loadings higher than 0.7 are selected, for PC3 parameters with loadings higher than 0.4 are selected. Percentages indicate the explained variance on each PC.

Parameter	**PC1 (33%)**	**PC2 (25%)**	**PC3 (8%)**
PC1			
Gape Period	0.970	0.168	0.017
Head Elevation Period	0.970	0.135	0.012
Head Elevation Moment	0.968	0.166	0.002
Food Period	0.968	-0.062	0.019
Food 2 Moment	0.965	0.136	0.027
Gape 2 Moment	0.963	0.219	0.025
Lower Bill 2 Moment	0.963	0.209	0.059
Lower Bill Period	0.959	0.164	-0.023
Difference Head Elevation	0.741	-0.617	-0.145
Gape 1	0.734	-0.462	-0.088
PC2			
Gape 2	-0.052	0.914	0.051
Neck Neck Moment	0.361	0.885	0.072
Neck Neck Period	0.364	0.877	0.085
Throat Moment	0.421	0.860	0.168
Gape Level	0.200	0.829	-0.073
Lower Bill at Gape 2	-0.482	0.804	0.203
Head X Moment	0.514	0.748	0.017
Lower Bill Level	-0.142	0.721	0.410
PC3			
Prokinesis at Gape 2	0.034	-0.154	0.744
Prokinesis at Gape 1	-0.429	0.143	0.639
Lower bill at Gape 1	-0.041	-0.004	0.513
Rhynchokinesis at Gape 2	-0.107	0.000	-0.474
Prokinesis Level	-0.635	0.089	0.469
Difference Neck Neck	0.230	0.390	0.437
Food Level Std	0.028	-0.298	-0.427
Food Level	0.457	-0.317	-0.419

variation (ANOVA, food-type 2: df=86, p=N.S.; food-type 3: df=89, p<0.05; food-type 4: df=168, p<0.05; food-type 5: df=100, p<0.05). Angular measurements were also used to test the response of the prokinetic and the rhynchokinetic movement during the feeding cycle. Prokinetic movement showed a pattern similar to the lower bill movement but with a much smaller amplitude (Fig. 7.9). However, the prokinetic movement pattern is not significant for any food-type or drinking (ANOVA, food-type 2, df=87, p=N.S.; food-type 3: df=89, p=N.S., food-type 4: df=167, p=N.S.; food-type 5: df=102, p=N.S.). The rhynchokinetic movement patterns can be clearly recognised except in the drinking behaviour (Fig. 7.9). The rhynchokinetic movement is only significant for the two largest food-types: small apple (food-type 2) and pellets (food-type 3)(ANOVA, food-type 2: df=84, p<0.05; food-type 3: df=88, p<0.05; food-type 4: df=166, p=N.S.; food-type 5: df=98, p=N.S.). In table 7.8 the maximal changes in the means angles of the different types of kinesis are given which shows an increase in cranial kinesis with an increase in food size.

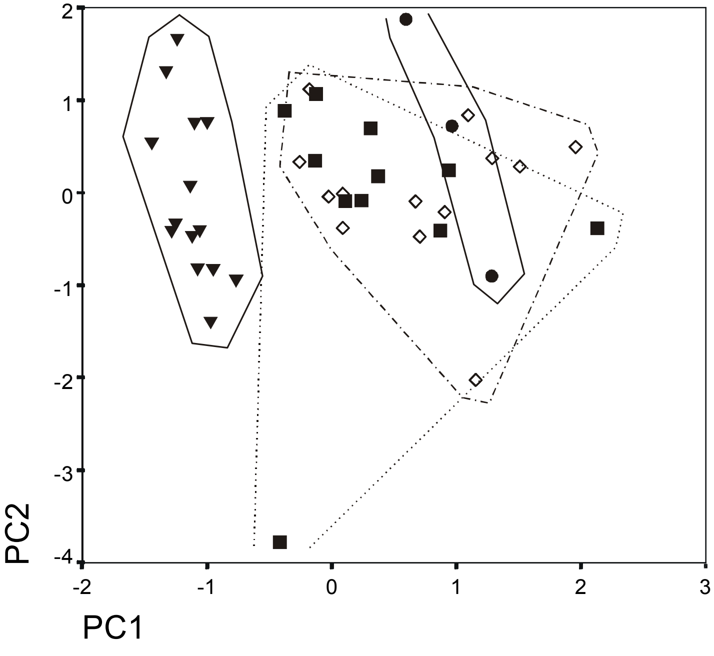


Figure 7.8. Plot of PC1 against PC2 of the analysis of food types only. Different food types are represented by different markers, and groups of a single food type are outlined. Circles: large apple, closed squares: small apple, open squares: pellets, triangles: seeds.

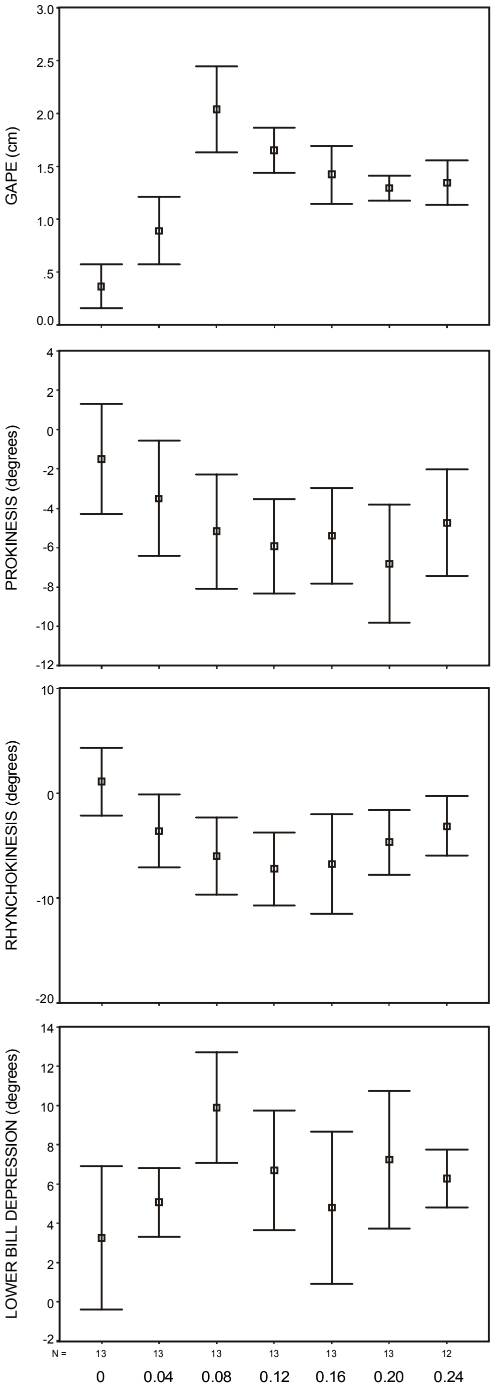


Figure 7.9. Kinesis in the upper bill of the Rhea. Squares indicate mean values (n=13) for each parameter at a certain time for the peck up phase with a medium sized food type. (3: pellets). Vertical lines indicate 95% confidence intervals. The gape is represented as the distance between the bill tips. Prokinesis, rhynchokinesis and lower bill depression are represented by change in angles (no absolute values). Negative angles for prokinesis and rhynchokinesis indicate elevation of the upper bill. Lower bill depression is represented by positive values.

Discussion

Feeding behaviour has been described for a large number of neognathous birds. In a comparative study a general feeding sequence has been described for the neognathous birds (Zweers *et al.*, 1994). Zweers and co-workers describe the most general form of avian feeding as pecking behaviour. This general pecking behaviour is divided into a number of elements. The first elements are preliminary head fixation, preliminary head approach and final head fixation. None of these elements are observed in the Rhea. A number of elements of the general neognathous pattern are characteristic for the feeding behaviour of the Rhea also. These elements are: 1) final head approach, 2) catch at jaw tips, 3) stationing and repositioning, 4) catch at jaw tips, 5) intra-oral transport (‘Catch & Throw’), 6) intra pharyngeal transport.

The complete intra-oropharyngeal phase as described for palaeognathous birds is

Table 7.8. Maximal change in mean of all feeding cycles of kinesis-parameters. Changes in gape are given in centimetres (cm), all other parameters are given in degrees (dg). Values between brackets indicate non-significant changes. L.=lower, Pro.=Prokinese, Rhyncho.=Rhynchokinese

Food	n	Gape (cm)	L. Bill (dg)	Pro. (dg)	Rhyncho. (dg)
Small Apple	11	1.8	(5.4)	(1.1)	10.1
Pellets	13	1.7	6.6	(3.7)	7.1
Seeds	12	0.9	4.2	(1.1)	(2.9)
Water	7	3.7	24.5	(6.6)	(4.3)

achieved by a single ‘Catch & Throw’ movement in the Rhea. This is in large contrast with the complicated ‘Slide & Glue’ mechanism and complex swallowing to transport food through the oropharynx often used by neognathous birds. However, in the case of large food-items neognathous birds may also use a single ‘Catch & Throw’ movement, but still show complex intra-pharyngeal transport (Zweers *et al.*, 1994). The difference between the single ‘Catch & Throw’ movement of neognathous and palaeognathous birds is that the neognathous birds use this movement to transport the food-item onto the lingual base, while palaeognathous birds use it to transport the food-item to the area caudal to the lingual base. The palaeognathous single ‘Catch & Throw’ movement is accompanied by a large gape and a large depression of the tongue. This depression results in an enlargement of the buccal cavity, which facilitates transport of the food-item into the caudal part of the oropharynx. Recently, a tongue muscle, the *Nervus hypoglossus* [XII] innervated *Musculus geniohyoideus*, was described, which is only found in Palaeognathae (Müller & Weber, 1998). Note that this muscle should not be confused with the *Nervus glossopharyngeus* [IX] innervated *Musculus geniohyoideus* as described by Zweers (1974) in the duck. The muscle described by Müller and Weber runs dorsal from to the *Musculus intermandibularis* from the *Rostrum mandibulae* to the ventral and latero-rostral side of the ceratobranchiale. The orientation of the muscle is such that it contributes to the large depression of the tongue during the characteristic ‘Catch & Throw’ movement in Palaeognathae. When the lower bill is slightly depressed and the *Musculus geniohyoideus* activated, it will pull the tongue ventro-rostral and will thereby strongly depress the mouth floor and enlarge the pharyngeal cavity. The pharyngeal cavity will become larger when the lower bill is more depressed. This mechanism is confirmed by roentgen-film observations that show protraction of the tongue during gaping (Tomlinson, 1997). Tomlinson (1997) also showed that the final transport of the food-item into the oesophagus is achieved by a single retraction of the tongue and larynx. The protraction/depression of the tongue during the ‘Catch & Throw’ movement and the retraction during swallowing are the only functions of the tongue during feeding. This indicates a difference from neognathous birds in which the tongue is often used during intra-oral transport (Zweers *et al.*, 1994).

To determine whether the feeding behaviour of the Palaeognathae is derived or primitive within modern birds a comparison must be made with the general feeding patterns found in other tetrapods. The method of feeding highly depends on the presence of a well-developed lingual apparatus. If a well-developed lingual apparatus is absent two main types of non-lingual feeding are present within the tetrapods: inertial feeding and the feeding pattern observed in snakes (de Vree & Gans, 1994). Avian 'Catch & Throw' is an inertial feeding technique, while the feeding pattern of snakes consists of left-right alternating mandibular retractions. Inertial feeding is found in many species of reptilia and can be considered the most general pattern of non-lingual feeding. Comparison of the feeding behaviour of the Rhea with the nearest living sister group of birds, the crocodilians, shows that the feeding behaviour of the Rhea is more similar to reptilian inertial feeding than the general feeding pattern of neognathous birds as described by Zweers *et al.* (1994). Crocodilian feeding is also divided in a number of elements: 1) killing/crushing, 2) transport and 3) swallowing (Cleuren & de Vree, 1992). These three elements can easily be compared with the different elements of the general avian pecking behaviour. The killing/crushing element is comparable to the grasp at the bill tips and repositioning. The transport phase can be compared to the intra-oral transport, and finally swallowing with intra-pharyngeal transport.

The similarity between the transport and swallowing phase in crocodilians and the intra-oral and intra-pharyngeal transport phases in the Rhea is remarkable. In crocodilian intra-oral transport the tongue elevates the food-item until it presses against the palate. Then a cycle occurs which is similar to a 'Catch & Throw' movement. The gape is rapidly increased, while simultaneously the tongue pushes the prey slightly upward. Then the cranium is lifted and moved forward and the tongue is depressed. In crocodilian feeding, tongue depression also results in a larger buccal cavity, which facilitates the transport of the food-item by inertial forces. The final transport of the food-item into the oesophagus in palaeognathous birds is also very similar to the crocodilian feeding behaviour. In the Rhea this transport is achieved by a retraction of the hyolingual apparatus (Tomlinson, 1997), similar to the transport in crocodilians.

For neognathous birds a general drinking pattern is also postulated (Zweers, 1992). This pattern consists of the following elements: 1) the fixation in which the bird orientates its head, 2) the down-stroke in which the head is lowered towards the water, 3) the immersion in which the actual water intake takes place, 4) the upstroke in which the head is positioned in such a way that gravitational forces facilitate transport of the water from the pharynx into the oesophagus (swallowing). In the general neognathous pattern (Zweers, 1992) the immersion phase can be divided into three elements that are different in the Rhea. In the first sub-phase (beak tip adhesion) the head is fixed and water enters the bill due to adhesion. In the Rhea there is no stationary phase, and in the immersion phase the bill is widely opened which makes adhesion forces negligible. The second phase in the general neognathous pattern is the intra-oral transport, which always includes pro- and retraction of the tongue. In the Rhea no tongue movement was observed in this phase and the main mode of intra-oral transportation is by means of a scooping motion of the head. In the third or storage phase, which is similar in

Palaeognathae and Neognathae, the water is kept in the oropharynx while the head is lifted during the upstroke.

The upstroke in the general pattern is also divided into subsequent elements: 1) the head elevation, also found in Rhea, 2) an elevation stop in combination with a swallowing cycle and 3) tipping up in which only the tips of the bill are elevated and gravitational forces transport the water into the oesophagus. In the Rhea we found no clear elevation stop. The head elevation movement gradually turns into a low amplitude tip-up phase in which a single protraction of the tongue facilitates the movement of the water into the oesophagus.

Although several differences in the feeding behaviour of Palaeognathae and Neognathae can be determined, most of them are reductions relative to the general neognathous feeding pattern. This indicates that no additional or different functional demands for the palaeognathous PPC can be expected relative to those of neognathous birds.

The food preferences of the Greater Rhea in the wild (Martella *et al.*, 1996) suggest that no fundamentally different feeding behaviours will be performed, than the ones analysed in our study. The feeding and food-acquisition behaviour of all Palaeognathae, except the Kiwi (*Apteryx* spec.), can be described as browsing. The diet consists of a wide variety of food-items, but is mainly vegetarian (Mosa, 1993; Martella *et al.*, 1996; Quin, 1996). There are some differences between the diets of the various palaeognathous species but these seem due to food availability, and not to preference or performance. This indicates that the main addition to our analysis might be that the Palaeognathae pull leaves of plants, which may add additional functional demands during food-acquisition. The removal of leaves is mainly achieved by neck motion, which generates external forces that will open the upper bill. These external forces might indicate a difference between the Palaeognathae and Neognathae but are not related to active cranial kinesis as found in neognathous birds.

The feeding behaviour of the Rhea resembles the feeding behaviour of the crocodilians, and lacks certain elements found in the general feeding pattern of neognathous birds. This suggests that the feeding behaviour of the Rhea is either primitive within birds or highly adapted to inertial feeding. A large problem in determining whether a feeding pattern is primitive or derived is that feeding is highly flexible and adaptive (de Vree & Gans, 1994). However, lingual feeding is found in the more primitive amphibians and inertial feeding is only found in cases where the tongue serves another function (de Vree & Gans, 1994). It is suggested here that lingual feeding is the most primitive avian feeding mechanism and we therefore conclude that the feeding behaviour of the Rhea should be regarded as general tetrapod inertial feeding which has evolved many times independently within vertebrates (de Vree & Gans, 1994).

The drinking behaviour of the Rhea lacks the tongue movement present in neognathous drinking. Although this very simple movement pattern suggests a basal position of the palaeognathous drinking behaviour, the absence of tongue movement may be the consequence of a reduction in size of the tongue in relation to an optimal ‘Catch & Throw’ feeding behaviour.

Our study showed that cranial kinesis in Palaeognathae is not limited to a single hinge or narrow bending zone. Kinesis is found both between the rostral and caudal part of the upper bill, and to a lesser extent between the caudal part of the upper bill and the cranium. Although the type of cranial kinesis of Palaeognathae has been described as central rhynchokinesis (Zusi, 1984) this study indicates that the movement in the upper bill of Palaeognathae is neither rhynchokinetic nor prokinetic but is flexible over its full length. The elevation amplitude of the bill tip relative to the cranium in the Rhea is similar to the elevation of the upper bill as found in prokinetic neognathous birds (approximately 5-10 degrees; Kooloos & Zweers, 1989; Heidweiller & Zweers, 1990; van den Heuvel, 1992). Because the bending zone is large in the Rhea these elevation angles are only achieved at the very tip of the upper bill. The actual elevation angle of the upper bill will gradually decline more caudally in the upper bill.

One hypothesis about the role of rhynchokinesis states that it can improve the holding of food-items as found in certain Charadriiformes (Zusi, 1984). These birds sometimes depress their upper bill tip around a food-item to improve holding. No upper bill depression is observed in the Rhea, which indicates that this cranial kinesis is not used in this way in Palaeognathae.

According to some authors cranial kinesis is the key character that played a role in the large radiation of birds. (Zweers *et al.*, 1997; Zweers & Vanden Berge, 1997b). In their view cranial kinesis has been a key-factor that resulted in the survival of certain species of birds during the mass-extinction at the Cretaceous-Tertiary transition. During this transition many non-kinetic species became extinct while the kinetic species survived. Zweers *et al*. assume that cranial kinesis resulted in the release of the horizontal component of the *Musculus pterygoideus* resulting in extra biting force. This extra biting force should increase the capacity to acquire food during the K-T transition. One of the groups that is assumed to have benefited from the cranial kinesis are the Palaeognathae. A previous study (Chapter 6) showed that the contribution of cranial kinesis to improved food manipulation is very limited.

Alternative hypotheses about the function and the origin of the palaeognathous PPC configuration have been postulated. The palaeognathous PPC is more robust than the neognathous configuration. One hypothesis states that the special morphology of the palaeognathous PPC is an adaptation to the large impact forces generated when the bill hits the ground during pecking (Bock, 1963). However, in our analysis it was found that impact forces on the bill are relatively low, which indicates that the birds are probably capable of reducing the impact force by reducing the speed at which the ground is hit. Large external forces from hitting the ground are therefore not very likely. During grazing, however, dorso-ventral forces reaction forces are present on the upper bill. It is possible that the morphology of the upper bill of the Palaeognathae is adapted to oppose these reaction forces.

Three hypotheses about the origin of the Palaeognathae agree with the assumption that feeding behaviour is primitive within this group. The first hypothesis is the most widely accepted one on the evolution of the Palaeognathae. It states that the Palaeognathae are the oldest offshoot in the phylogeny of modern birds (Bock, 1963; Meise, 1963, Parkes & Clark, 1966; Cracraft, 1974; de Boer, 1980; Prager and Wilson, 1980; Sibley & Ahlquist, 1981; McGowan, 1984; Feduccia, 1985; Handford & Mares, 1985; Elzanowski, 1986, Houde, 1986, Bledsoe, 1988; Caspers *et al.*, 1994, Lee *et al.*, 1997). The second hypothesis states that Palaeognathae have evolved through neoteny from a flying ancestor (de Beer, 1956). The hypothesis on the neotenous origin of the Palaeognathae is recently revived by physiological/ontogenetic data (Dawson *et al.*, 1994) and molecular systematics (Mindell *et al.*, 1997; Härlid & Arnason, 1999). The physiological/ontogenetic experiments showed that induced neoteny in neognathous birds results in a morphology of the PPC that was similar to that of the Palaeognathae, while the molecular systematic data show a derived position of the Palaeognathae within the Neognathae and not a basal position of the Palaeognathae. A third hypothesis on the origin of the special morphology of the palaeognathous PPC suggests that the morphology of the extant Palaeognathous PPC is the result of the continuous reduction of bony and ligamentous elements in the lateral aspect of the skull (Gussekloo and Zweers, 1999). Although birds in general have less bony and ligamentous elements in the lateral aspect of the skull than closely related groups such as dinosaurs and other reptiles, Palaeognathae have even less than most birds. Compared to Neognathae, Palaeognathae lack a clear *Ligamentum postorbitale* and the lateral bar of the upper bill (Bock, 1964; Zusi, 1984). The reduction of these elements might have resulted in a relatively unstable configuration of the upper bill, especially when these birds increased in size. This unstable upper bill configuration was reinforced by creating a more rigid ventral plane of the upper bill. This plane is mainly formed by the PPC. The reinforcement of the ventral plane of the upper bill limited the active kinesis in the upper bill. This limited kinesis without clear bending zones might be an adaptation to protect the very well developed olfactory organs in the nasal cavity. Absence of bending and the presence of the broad PPC might protect the nasal cavity from movement damage.

Although our results cannot discriminate between these three hypotheses it is clear that the Palaeognathae have a very typical feeding behaviour with very little variation that indicates that the feeding behaviour is either basal within birds or highly adapted to ‘Catch & Throw’ feeding. The role of active cranial kinesis in palaeognathous feeding behaviour is not very large, and it can also not be described as rhynchokinesis since flexibility is observed throughout the upper bill. This also points at a primitive condition, which limits the discussion of the origin of the Palaeognathous feeding apparatus to the question, how to discriminate between primitive and neotenous conditions.

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 &

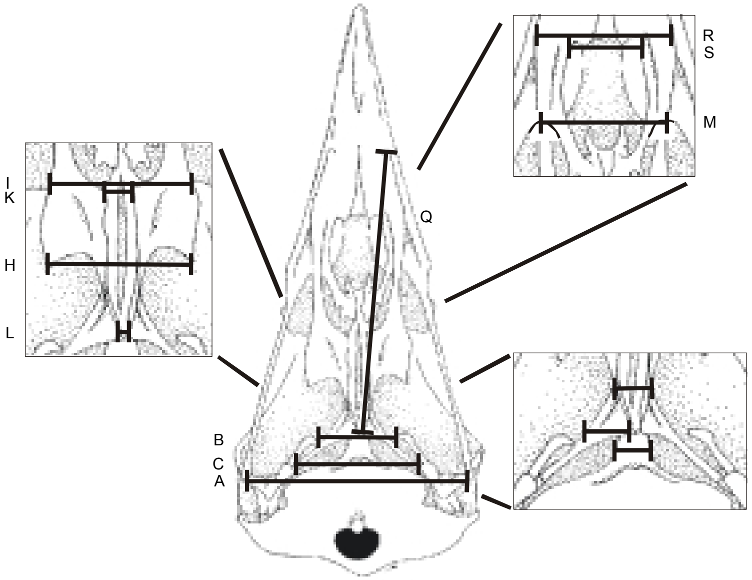


Figure 8.1. Skull of the crow (Corvus corone) in ventral view. Inserts are enlargements of areas indicated by the lines. Letters refer to characters in table 8.1. The characters of the vomer cannot be represented since the vomer is reduced in this species. Other characters omitted from this figure for 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.

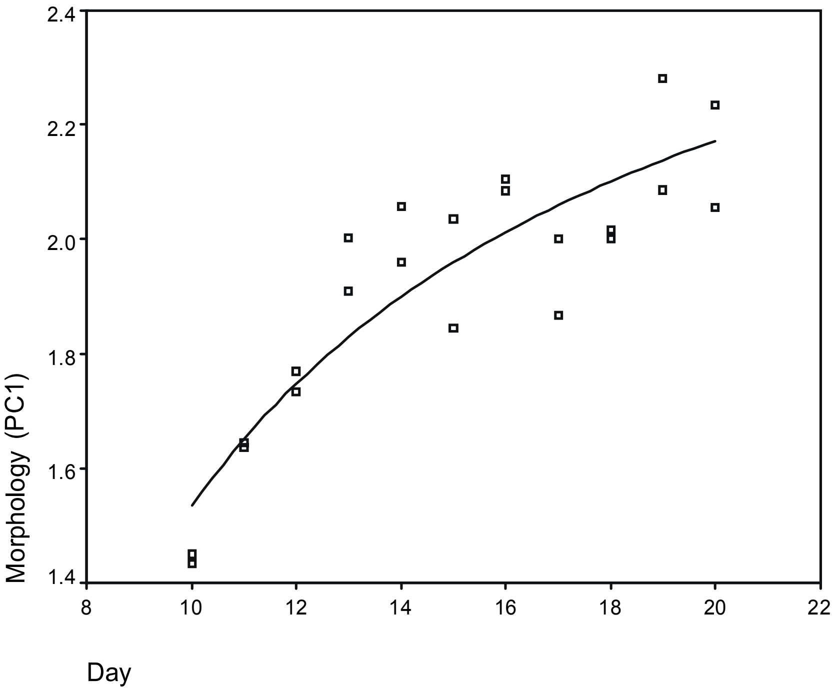


Figure 8.2. Morphology (described by the PC1) versus day of development for a neognathous bird (Gallus gallus).

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

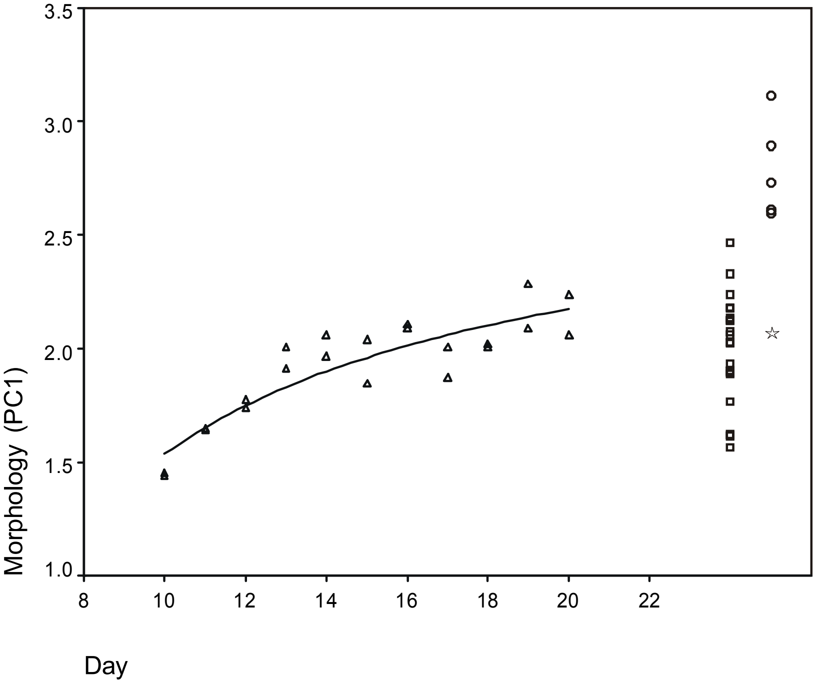


Figure 8.3. Morphology (described by the PC1) versus day of development. Far clarity adult specimens are displayed directly left of the graph. Triangles indicate embryonic neognathous birds, squares indicate adult neognathous birds, circles indicate palaeognathous birds, and the star indicate a representative 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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CHAPTER 9

SUMMARY & GENERAL DISCUSSION

General Summary

The evolutionary history of the Palaeognathae has puzzled biologists for more than a century. The main questions have always been the monophyly of the taxon, and the question whether the Palaeognathae are primitive or derived within modern birds. The first problem seemed to have been solved since the development of molecular phylogenetic techniques. Almost all molecular phylogenetic studies point in the direction of a monophyletic origin of the Palaeognathae. The second dispute in the evolutionary theory about the Palaeognathae is the point of origin of the taxon. The most generally accepted hypothesis claims that the Palaeognathae are the most basal group within modern birds. An opposing hypothesis claims that the Palaeognathae are derived within modern birds and that their characters have evolved through neoteny.

In this thesis an attempt is made to solve the question where in the avian phylogeny and how in avian evolution the bifurcation between the Neognathae and Palaeognathae took place. This is done by analysing one of the most characteristic features of the Palaeognathae: the palaeognathous Pterygoid-Palatinum Complex (PPC). The PPC in neognathous birds is part of the mechanism that elevates the upper bill. This function has, however, never been confirmed for the Palaeognathae, nor is it clear what selective forces have resulted in the special morphology of the palaeognathous PPC.

Although the Palaeognathous PPC was already recognised at the end of the 19th century (Huxley, 1867), some authors disputed the existence of the character (McDowell, 1948). In chapter two it was shown that the palaeognathous PPC is indeed a character complex that is clearly different from the neognathous condition and can be quantitatively described. An outgroup analysis showed that the palaeognathous condition is more primitive than the neognathous condition. The main characters of the PPC that differ between the palaeognathous and neognathous PPC are: a large *Processus basipterygoideus*, a short *Processus orbitalis quadrati*, a broad and rostrally situated pterygoid-palate articulation, a broad vomer and broad pterygoids. In general the five qualitative characters given by Bock (1963) describe the palaeognathous palate accurately. We can therefore conclude that the palaeognathous PPC is a true character that distinguishes the Palaeognathae from the Neognathae. Our study of the PPC showed that the palaeognathous condition is only found in the Palaeognathae, also indicating a monophyletic origin of this condition.

This allows us to consider the Palaeognathae to be monophyletic and their characters specific for this group. This allows us to compare our findings about the palaeognathous PPC with the evolutionary pathway as proposed by Zweers *et al.* (1997; see also Zweers & Vanden Berge, 1997). To do this a functional analysis is made of the PPC with special attention to the proposed linkage between the morphology of the palaeognathous PPC and central rhynchokinesis.

To determine the relation between PPC morphology and the type of kinesis, the morphology of a neognathous rhynchokinetic bird was investigated. To distinguish between neognathous characters and rhynchokinetic characters, this neognathous rhynchokinetic bird was first compared with the general prokinetic morphology. In chapter three an analysis is made of the morphological characters and feeding patterns of the Red Knot (*Calidris canutus*), a specialised rhynchokinetic prober. This analysis showed that features that can be related to rhynchokinetic feeding behaviour are mostly found in the bill itself. The main characters are the clearly recognisable flexible zones in the dorsal and ventral bars and the uncoupling of the dorsal and ventral bar through a schizorhinal nostril. Morphological differences in the PPC of the rhynchokinetic Red Knot compared to that of prokinetic birds were not found, indicating that the type of kinesis does not have a large effect on the configuration of the PPC. This might indicate that there is no relation between the palaeognathous PPC and central rhynchokinesis.

Although no large differences could be found between the PPC morphology of neognathous prokinetic and neognathous rhynchokinetic birds, it is possible that the special PPC of the Palaeognathae is the result of a difference in function between the Palaeognathae and the Neognathae. To test this we determined the movement pattern of the PPC during elevation of the upper bill. To give an accurate and complete description of the relatively small movements, the displacement of the PPC had to be determined in three-dimensions. For this three-dimensional analysis a new roentgen-stereophotogrammetry technique was developed.

Chapter four describes the method that was used to determine the displacement of bony elements in skulls with different types of cranial kinesis. The method is a combination of roentgen-photography and stereophotogrammetry. Stereophotogrammetry is used to determine three-dimensional co-ordinates of object markers from at least two photographs of the object from different angles. Normally these two photographs are obtained by moving the camera around the object or by using two cameras. Neither of these methods could be used in combination with roentgen-photography since only one roentgen-source was available and movement of a roentgen-camera leads to a change in the interior orientation of the camera. In roentgen-photogrammetry the roentgen-source and the film are not connected and movement of the camera (source and receiver) results in a change of the configuration of the camera. Any change in configuration decreases the accuracy of the calculations. In collaboration with the Delft University of Technology a new method was developed to overcome these problems. A single roentgen-source was used, and instead of moving the camera, the object was moved within the roentgen-bundle, and photographed from six different angles. From these radiograms the co-ordinates of markers are calculated with an accuracy of 0.12 mm.

In chapter five the stereo roentgen-photogrammetry method was used to determine the displacement of several bony elements of the jaw apparatus, including the PPC, after elevation of the upper bill in skulls of five different species with prokinetic (*Corvus corone*), and rhynchokinetic skulls (*Calidris canutus*, *Struthio camelus*, *Dromaius novaehollandiae*, *Rhea americana*). It was shown that Bock's qualitative description (1964) of the movement of the PPC during bill opening was accurate, and that no large differences are present in the movement patterns of birds with different types of kinesis. This indicates that the functional demands for movement acting on the PPC for elevating the upper bill are similar for all types of kinesis.

In the three-dimensional kinematic analysis elevation was imposed by applying external forces. Since any structure will bend if sufficient force is applied, it was investigated whether the bill morphology of the Palaeognathae was adapted to rhynchokinesis. In this analysis we used the bill characters found in the distal rhynchokinetic Red Knot (*Calidris canutus*) to define a rhynchokinetic bill. The morphological adaptations that have to be present for rhynchokinesis are: clear bending zones in the dorsal and ventral bars, uncoupling of the dorsal and ventral bar, and sufficient muscle force to elevate the upper bill. In addition we investigated whether the force of the pterygoid muscles contribute to biting force as suggested by Zweers *et al.* (1997) for the palaeognathous ancestral trophic system. We found that uncoupling of the dorsal and ventral bar of the upper bill is present. However, no clear bending zones could be detected in the bars of the upper bill. Some thinner zones are present in the bars of the upper bill, but the position of these zones differs from the position where bending is observed. The analysis of muscle force showed that the force of the *Musculus protractor pterygoideus et quadrati* is sufficient to elevate the upper bill. The analysis also showed that the configuration of the muscles and the skull of the Palaeognathae results in very low biting force. A remarkable feature of the Palaeognathous skull is the presence of the large *Processus basipterygoideus*. It was found that this element, in combination with the large *Musculus pterygoideus* can act as a stabilising mechanism, which prevents the upper bill from being depressed or elevated by external forces. Large elevation forces may occur when the birds pull leaves from plants. These findings indicate that the upper bill of the Palaeognathae is not ‘adapted’ to active rhynchokinesis, but that movement in the upper bill occurs as a result of intrinsic flexibility in the bone itself.

In chapter seven the feeding behaviour of the Palaeognathae was studied. The transport phase of the feeding pattern is a very simple inertial ‘Catch & Throw’ type. Drinking behaviour is basal and consists of a scooping movement followed by a low-amplitude tip-up phase only. Cranial kinesis is limited and occurs only during gaping and is always orientated dorsally (elevation). It was also found that bending occurs over the full length of the upper bill, but is most prominent at the bill tip. The grasping and transport phases in palaeognathous feeding behaviour strongly resembles that of neognathous birds and there is no indication that Palaeognathae experience other selective forces on the PPC than Neognathae as a result of the feeding behaviour investigated. Furthermore, the Palaeognathae are not able to generate large biting forces in their feeding behaviour.

In chapter eight the hypothesis about the neotenous origin of the Palaeognathae was tested. A numerical method was used to compare the PPC of adult palaeognathous species with the PPC of stages in the development of a neognathous bird. This showed that there are large differences between the morphology of the Palaeognathae and the embryonic stages of the neognathous bird, indicating a non-neotenous origin of the Palaeognathae.

Avian Evolution during the Cretaceous

Our findings will be used to test the evolutionary pathway proposed by Zweers *et al.* (1997; see also Zweers & Vanden Berge, 1997). I consider the origin of all birds to be within the Coelosauria and probably closely related to the Dromaeosauria (Padian & Chiappe, 1998). The first step in the direction of the avian feeding mechanism is the fenestration of the skull and reduction of the bars in the lateral aspect of the skull as described by Zweers *et al*. (1997). This reduction resulted in a flexible skull without a mechanism for cranial kinesis. The next step in the evolution of birds is assumed to be the detachment of the palate, which was achieved in three different ways resulting in a trifurcation of evolutionary pathways. The three lineages are described as a non-kinetic Dromaeosauro-/Archeopterygo-/Enantiornithomorf lineage, a pre-rhynchokinetic Ornithomimo-/Hesperornitho-/Ratitomorph (now called Palaeognathous) lineage and a (pre-)prokinetic Troodonto-/Neognathomorph (now called Neognathous) lineage. Because modern birds are only found in the latter two, we will focus on these two lineages. This trifold radiation was assumed on the basis of the reduction of the pterygoid and the capacity to transfer force between upper bill and quadrate. The main difference between the proposed Palaeognathous lineage and the Neognathous lineage described by Zweers *et al.* (1997) is the presence of a vomeral-maxillary bar. Other characters discriminating between the two lineages are found in the pterygoids, but these do not differentiate the Palaeognathous and Neognathous lineages completely. In both lineages two pterygoids are present during development, of which the rostral part becomes either incorporated in the palato-vomeral-maxillary apparatus or disappears completely. Remarkable is also that the primary division described by Zweers *et al.* (1997) results in akinetic species in all three lineages, and that in each lineage an independent development of kinesis occurs. Our study (Chapter 5) showed that in each of the proposed kinetic lineages both the role and position of bony elements (except for the vomer) and the movement pattern of the PPC are very similar. This indicates that the detachment of the palate has occurred earlier in evolution resulting in a bifurcation in a non-kinetic and a kinetic lineage. With this modification, the transformation pattern of Zweers *et al.* (1997) represents the classical situation in which the Palaeognathae are basal within modern birds, and the sister group of the Neognathae (Cracraft, 1986; Sibley & Ahlquist, 1990). Based on the findings of Cooper & Penny (1997) the kinetic-lineage must have originated during the Cretaceous, followed by a very early bifurcation between the Palaeognathae and Neognathae.

The K-T transition

The next step in the evolutionary pathway is the transition at the K-T boundary. A large ecological effect during the K-T transition was a change in climate what resulted in a decline in food resources. For the palaeognathous lineage Zweers *et al.* (1997) described the available food resources as tough foliage that requires large biting forces, a highly modifiable rhamphotheca and hetereocoelic vertebrae. In our analysis we mainly focused on biting force since this is the functional demand that directly acts on the jaw-apparatus. A large biting force is thought to arise from a kinetic skull, a detached palate (PPC), and an extra force-component from the *Musculus pterygoideus*. After the detachment of the palate, the *Musculus pterygoideus* is assumed to add an additional component to the closing forces on both the upper and lower bill.

This seems incorrect for the palaeognathous lineage. However, the feeding behaviour as described for the extant palaeognathous birds seems fit for eating tough foliage also indicating an alternative solution. For this feeding behaviour the birds do not use large biting force for their food-uptake (Chapter 7) but have developed a mechanism that resists upper beak elevation due to external forces. The overall morphology also indicates that the Palaeognathae are not adapted to rhynchokinesis. Most important it was shown that the well-developed pterygoid muscles in the Palaeognathae do not contribute significantly to total biting force (Chapter 6). I therefore conclude that the hypothesis of Zweers *et al.* (1997) that the *Musculus pterygoideus* in combination with a moveable increases biting force is incorrect for the Palaeognathae. As observed correctly by Zweers *et al.* (1997) the additional force from the pterygoid muscles acts on both the upper and lower bill. However, due to the very ineffective position of the pterygoid muscles on the lower bill, the contribution to upper jaw depression is much higher than the contribution to lower jaw elevation. The depression of the lower bill must therefore be counteracted by an activation of the lower jaw adductor muscles. If therefore the pterygoid muscles become larger, the adductor muscles have to become larger as well, to counteract the forces of the pterygoid muscles.

Although biting force does not increase it is not impossible that the palaeognathous PPC configuration made the transition of the K-T boundary possible but for a reason different from biting force. If the ancestral Palaeognathae fed on tough foliage during the K-T transition it is likely that they used the same mechanism as the extant Palaeognathae. These birds remove leaves from plants, or from the ground, by a caudal pull of the vertically orientated head (Chapter 7). These movements result in large external forces on the upper bill, which might result in opening of the upper bill. The large *Musculus pterygoideus* can be used to counteract these external forces. To prevent the risk of overcompensation, resulting in depression of the upper jaw, the *Processus basipterygoideus* is present, which is used to ‘block’ the depression of the upper bill (Chapter 6).

The fact that the upper bill has to be stabilised by a muscle indicates that the ancestor of the Palaeognathae must have had a kinetic skull, and that the upper bill was stabilised later in evolution. The main question that results from this conclusion is whether the ancestor of the Palaeognathae was prokinetic or rhynchokinetic. As mentioned before, the morphology does not confirm a rhynchokinetic condition, since flexible zones are absent. However, the dorsal and ventral bar of the upper bill are uncoupled, but by a mechanism that is not found in other rhynchokinetic birds. The presence of the holorhinal nostril indicates that the palaeognathous mechanism has either evolved from an akinetic, or a prokinetic ancestor.

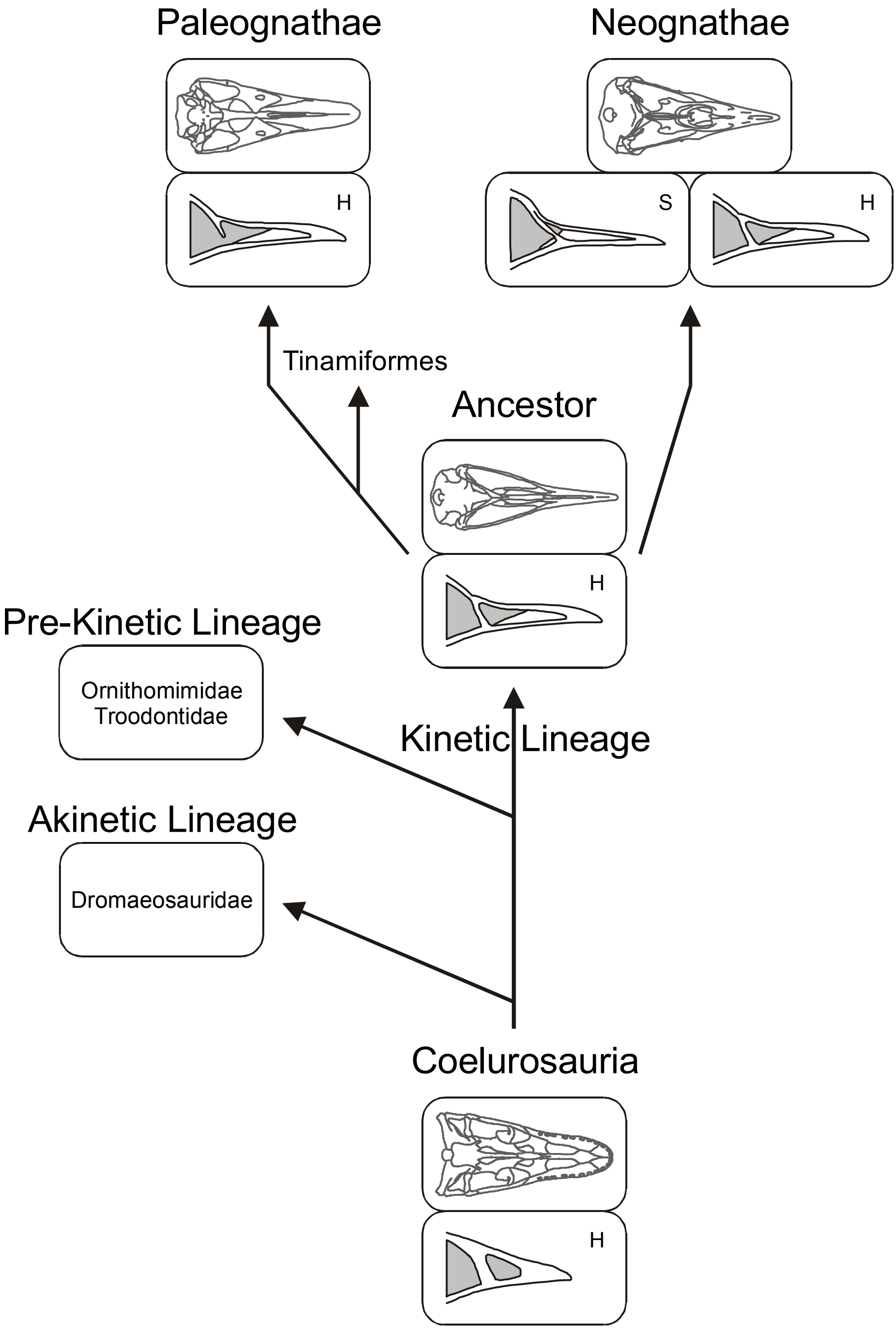


Figure 9.1. Caption on facing page.

An alternative hypothesis is that the holorhinal nostril is one of the characters that might have evolved through neoteny. In that case it is still possible that the ancestor of the Palaeognathae had a schizorhinal nostril and was rhynchokinetic. Our analysis of the PPC indicated that the PPC does not possess characters that are also found in embryological stages of neognathous development. I therefore reject the hypothesis about neotenous-origin of the palaeognathous PPC. It is still possible that characters such as flightlessness and downy feathers might be the effect of neoteny (de Beer, 1956).

The evolution of the Palaeognathous birds

In accordance with the findings described above a new evolutionary pathway for the Palaeognathae is postulated (Fig. 9.1). The different morphology of the Palaeognathae made it possible to resists external forces opening the upper bill during grazing on tough foliage. This adaptation made it possible for the Palaeognathous lineage to pass the K-T boundary confirming the hypothesis that the bifurcation between the Palaeognathae and Neognathae must have occurred in the Cretaceous period (Cooper & Penny, 1997; Zweers *et al.*, 1997). The similar movement patterns of the PPC suggest that the skull of the ancestral bird must have been kinetic with a detached palate. From this ancestral design two lineages evolved, the Palaeognathae and Neognathae. The main question is to determine the ancestral design. Since both the palaeognathous and neognathous lineage evolved directly from the ancestral design (neoteny did not play a role) all elements present in both lineages must have been present in the ancestral design. Therefore the ancestor of the modern birds must have possessed both a vomer and a *Processus basipterygoideus*. Since a holorhinal nostril is present both in the Palaeognathae and Neognathae it is expected that the ancestral bird also had a holorhinal nostril and was probably (pre-)prokinetic. The overall skull configuration of the ancestral type might resemble the morphology of the modern Tinamiformes. The skull morphology of the Tinamiformes possesses all elements of the Palaeognathous PPC, possesses a holorhinal nostril and a complete lateral bar. A diversification in feeding behaviour probably resulted in the bifurcation of the Neognathae and Palaeognathae. The palaeognathous lineage started feeding

Figure 9.1. (on facing page) Adapted evolutionary pathway of the Paleognathae based on the hypothesis of Zweers et al. (1997). Within the Coelurosauria first an akinetic lineage splits of, including the Dromaeosauridea. The other lineage has reduced cranial bars and is considered pre-kinetic. This latter lineage divides in a kinetic (Avialae) and a pre-kinetic lineage based on the detachment of the palate. The pre-kinetic lineage includes the Ornithomimidae and the Troodontidae. The ancestral design within the kinetic lineage has a moveable palate, a holorhinal nostril and is (pre-) prokinetic. Based on differences in food acquisition a bifurcation in the Paleognathae and Neognathae occurred. This last bifurcation most probably occurred before the Cretaceous-Tertiary boundary. Within the paleognathous lineage the Tinamiformes are probably the most basal group. H=holorhinal nostril, S=schizorhinal nostril.

on food-types that required large forces to acquire and process. Although I doubt that the Palaeognathae were the only group that fed on this food-type, I assume a different solution to the specific problems of feeding on these food-types between the two lineages. While in neognathous birds the tough food-items were probably sliced with the sharp edges of the rhamphotheca, these sharp ridges did not evolve in the Palaeognathae. To acquire food, the Palaeognathae used forces generated by the neck to pull off leaves and without the ability to reduce the food-items in size developed a feeding mechanism optimised for the intra-oral transport of large objects. The use of neck muscle force resulted in a secondary demand to strengthen the bill configuration, which was achieved by a strongly developed PPC. It can be hypothesised that the method of feeding, in which the neck produces the main forces to acquire food, has resulted in the increase in size of the Palaeognathae so that forces can become larger.

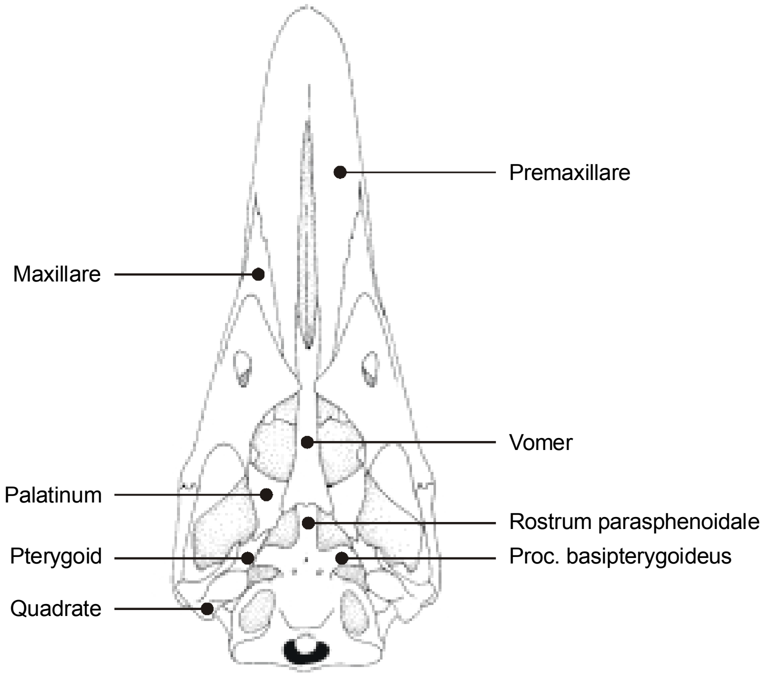
I therefore concur with the hypothesis of Zweers *et al.* (1997) that the Palaeognathae had a sufficient design to pass the K-T boundary. I also conclude that the Palaeognathae must have evolved before the K-T boundary from an already (pre-) prokinetic ancestor. Based on the presence of the large *Processus basipterygoideus* and the large *Vomer* the PPC must resemble a very basal condition. I conclude that the Palaeognathae are the most basal group in modern birds and the sister-groups of all other living birds.

CHAPTER 10

SAMENVATTING & DISCUSSIE

Samenvatting

De levende vogels worden verdeeld in twee grote groepen. De belangrijkste en grootste groep wordt de Neognathae genoemd en bestaat uit ongeveer 8000 soorten. De andere groep wordt de Palaeognathae genoemd en bestaat uit minder dan 100 soorten. De Palaeognathae bestaan uit twee groepen: de Ratites en de Tinamous. De Tinamous (Tinamiformes) zijn kleine, slecht vliegende, op kippen lijkende vogels, die voorkomen in Zuid-Amerika. De groep Ratites bestaat uit 10 levende soorten die verspreid over het zuidelijk halfrond voorkomen. Deze soorten zijn de Nandoe (*Rhea americana*) en Darwin nandoe (*Pterocnemia pennata*) uit Zuid-Amerika, de Struisvogel (*Struthio camelus*) uit Afrika, drie soorten Casuarissen (*Casuarius casuarius*, *C. bennetti*, *C. unappendiculatus*) uit Nieuw-Guinea en Noord-Australië, de Emu (*Dromaius novaehollandiae*) uit Australië en drie soorten Kiwi's (*Apteryx australis*, *A. owenii*, *A. haastii*) uit Nieuw-Zeeland.

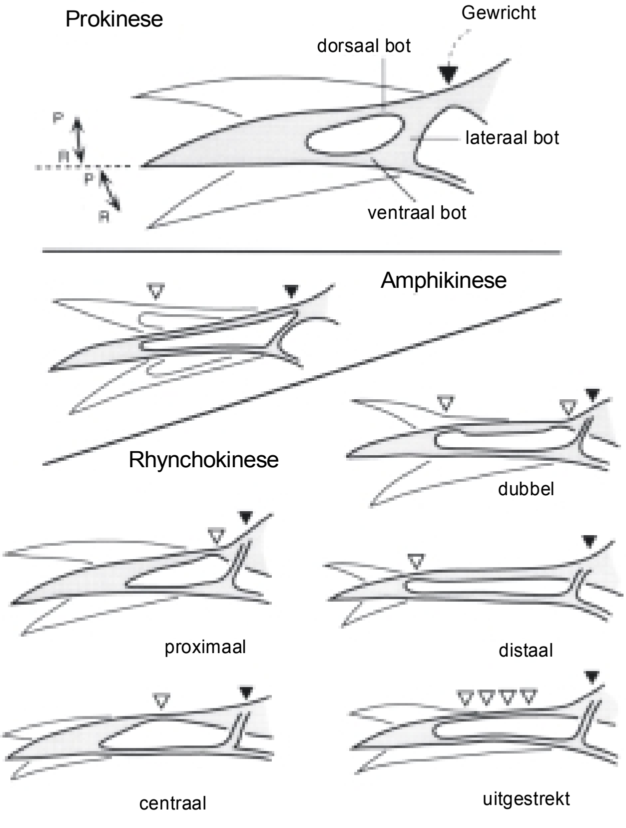


Figuur 10.1. Ventraal aanzicht (van onder) van het paleognathe Pterygoid-Palatinum Complex (PPC) van de Nandoe (Rhea americana).

Hoe en wanneer de Palaeognathae zijn ontstaan (de evolutie) is een groot probleem geweest voor biologen sinds de eerste keer dat de groep werd beschreven aan het einde van de 19e eeuw. De belangrijkste vragen in verband met de Palaeognathae betreffen de monofylie (alle soorten ontstaan uit één gemeenschappelijke voorouder-soort) van de groep, en de vraag of de Palaeognathae aan het begin of aan het eind van de stamboom van de levende vogels zijn ontstaan. Het eerste vraagstuk lijkt te zijn opgelost door middel van moleculaire fylogenetische technieken (stamboom reconstructie met behulp van DNA). Bijna alle resultaten van moleculaire fylogenetische analyses wijzen in de richting van een monofyletische oorsprong van de Palaeognathae (bv. Sibley & Ahlquist, 1990). Het tweede vraagstuk over de evolutie van de Palaeognathae gaat over de oorsprong van de groep. De meest geaccepteerde hypothese stelt dat de Palaeognathae de oudste vogelgroep is, gekenmerkt door primitieve kenmerken (bv. Cracraft, 1974). Daartegenover staat de theorie dat de Palaeognathae pas recentelijk zijn ontstaan, en dat deze schijnbaar primitieve kenmerken van de groep zijn ontstaan door neotenie (bv. de Beer, 1956). Dit betekent dat volwassen dieren kenmerken hebben van onvolwassen dieren, die ook wel primitief worden geacht. De donsachtige veren van volwassen Palaeognathae zouden hier een voorbeeld van kunnen zijn.

In dit proefschrift is een poging gedaan om de vragen te beantwoorden waar in de vogel fylogenie (stamboom), en hoe in de evolutie, de splitsing tussen de Palaeognathae en Neognathae heeft plaatsgevonden. Dit is gedaan aan de hand van een analyse van één van de belangrijkste kenmerken van de Palaeognathae: het palaeognathe Palatinum-Pterygoid Complex (PPC). Dit complex bestaat uit een aantal botten in de schedel die er bij palaeognathe vogels heel anders uitzien dan bij neognathe vogels (Fig. 10.1). Deze botten zorgen bij de Neognathae voor de beweging van de bovensnavel. De mogelijkheid om de bovenkaak (=bovensnavel) te bewegen (craniale kinese) is specifiek voor vogels en sommigen reptielen, voornamelijk hagedissen. Binnen de vogels is er een aantal verschillende vormen van kinese beschreven (Fig 10.2; Zusi, 1984). De belangrijkste, prokinese, wordt gekenmerkt door een gewricht aan de basis (kop-kant) van de snavel en een beweging van de volledige snavel. De op één na belangrijkste vorm van kinese is rhynchokinese, waarin het gewricht aan de basis van de snavel is gestabiliseerd en door middel van buigzones in de snavel de beweging van (een deel van) de snavel naar voren is verplaatst. Aan de hand van de positie van de buigzones wordt een aantal verschillende types onderscheiden: proximale rhynchokinese, met de buigzone bij de basis; distale rhynchokinese, met de buigzone bij het puntje van de snavel en centrale rhynchokinese, met het buigpunt in het midden van de snavel. Daarnaast kunnen er twee buigzones zijn (dubbele rhynchokinese) of één zeer lange buigzone (uitgebreide rhynchokinese). Opvallend is dat aan de hand van schedels voor alle Palaeognathae is beschreven dat ze centrale rhynchokinese bezitten. Een functie van het PPC in verband met craniale kinese is echter nog nooit beschreven voor de Palaeognathae en het is ook niet duidelijk welke selectieve krachten hebben geresulteerd in de speciale morfologie (vorm) van het palaeognathe Palatinum-Pterygoid Complex.

Hoewel het palaeognathe PPC al aan het eind van de 19^{e} eeuw voor het eerst werd beschreven (Huxley, 1867), zijn er auteurs die aan de echtheid van het kenmerk twijfelden (bv. McDowell, 1948). In hoofdstuk twee wordt aangetoond dat het palaeognathe PPC inderdaad een kenmerk is dat kwantitatief kan worden beschreven en dat het PPC van de Palaeognathae duidelijk afwijkt van dat van de Neognathae. Een fylogenetische analyse met behulp van een ‘outgroup’ (nauw-verwante, niet-vogelsoort) toont aan dat de Palaeognathae primitief zijn binnen de moderne vogels. De belangrijkste kenmerken van het PPC die aangetroffen worden binnen de Palaeognathae, in tegenstelling tot de Neognathae, zijn (Fig 10.1): een relatief grote *Processus basipterygoideus*, een relatief korte *Processus orbitalis quadrati*, een brede en een relatief naar voren gepositioneerde verbinding tussen het pterygoid en palatinum, een breed

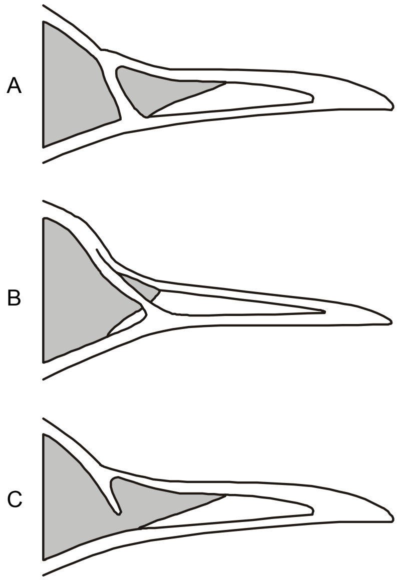


Figuur 10.2.Typen van craniale kinese in vogels (Aangepast van Zusi, 1984). P=protractie, R=retractie; Dichte pijlen wijzen naar het snavel-schedel gewricht, open pijlen wijzen naar de buigzones in de bovensnavel.

vomer en brede pterygoiden. Over het algemeen beschrijven de vijf door Bock (1963) gegeven kwalitatieve kenmerken het palaeognathae PPC goed en komen deze in grote mate overeen met de door ons gevonden kenmerken. We kunnen daarom concluderen dat het palaeognathe PPC een bestaand kenmerk is dat onderscheid maakt tussen Neognathae en Palaeognathae. Ons onderzoek van het PPC heeft aangetoond dat de palaeognathe configuratie van het PPC alleen binnen de Palaeognathae wordt gevonden, wat ook duidt op een monofyletische oorsprong van de Palaeognathae. Dit stelt ons in staat om de monofyletische oorsprong van de Palaeognathae te accepteren.

Vervolgens zijn onze vindingen omtrent het PPC vergeleken met de hypothese van Zweers *et al.* (1997; zie ook Zweers & Vanden Berge, 1997; voor een Nederlandse tekst zie ook Zweers, 1999) over de evolutie van vogels. Dit wordt gedaan aan de hand van een functionele analyse van het PPC met speciale aandacht voor de veronderstelde relatie tussen de morfologie van het palaeognathe PPC en centrale rhynchokinese.

Om de relatie tussen het PPC en de vorm van kinese te bepalen is de morfologie van een neognathe rhynchokinetische vogel onderzocht. Om onderscheid te kunnen maken tussen specifieke kenmerken van Neognathae en de specifieke kenmerken van een rhynchokinetische vogel is de morfologie eerst vergeleken met een algemene beschrijving van de morfologie van een neognathe prokinetische vogel. In hoofdstuk drie is een analyse gemaakt van de morfologische kenmerken en het voedselopname-gedrag van de Kanoetstrandloper (*Calidris canutus*), een gespecialiseerde distaal rhynchokinetische sondeerder (Deze soort prikt met zijn snavel in het wad om schelpen en kleine geleedpotigen te vinden). Deze analyse toont aan dat kenmerken die te maken hebben met rhynchokinese voornamelijk worden aangetroffen in de snavel zelf. Deze kenmerken zijn de duidelijk herkenbare buigzones in de dorsale (aan de rugzijde=boven) en ventrale (aan de buikzijde=onder) botelementen (Fig. 10.2) en de ontkoppeling van de beweging in de dorsale en ventrale botelementen door middel van een speciale (schizorhinale) configuratie van de botten rond het neusgat (Fig. 10.3). Morfologische verschillen in het PPC tussen de rhynchokinetische Kanoetstrandloper en een prokinetische



Figuur 10.3. Configuraties van neusgaten. Grijze gebieden zijn het inter-orbitaal en inter-nasale septum.
Neognath holorhinaal
Neognath schizorhinaal
Paleognath holorhinaal

neognathe vogel worden niet gevonden, wat er op duidt dat het type craniale kinese geen grote invloed heeft op de configuratie van het PPC.

Hoewel er geen grote verschillen in de morfologie van het PPC tussen neognathe prokinetische vogels en neognathe rhynchokinetische vogels konden worden gevonden, is het nog steeds mogelijk dat de speciale morfologie van het PPC in Palaeognathae het resultaat is van een verschil in functie van het PPC in Neognathae en Palaeognathae. Om dit te toesten is de beweging van de verschillende elementen van het PPC bepaald tijdens het openen van de bovensnavel. Om de geringe verplaatsing zo nauwkeurig mogelijk te bepalen, zijn ze in drie dimensies berekend. Om deze bepalingen mogelijk te maken is in samenwerking met de Technische Universiteit Delft een nieuwe röntgen stereo-fotogrammetriemethode ontwikkeld.

Hoofdstuk vier beschrijft de methode zoals deze is gebruikt om de verplaatsingen van botelementen in de schedel te bepalen. De methode is een combinatie van röntgen-fotografie en stereo-fotogrammetrie. Stereo-fotogrammetrie wordt gebruikt om driedimensionale coördinaten van object-markers te bepalen aan de hand van tenminste twee foto's van het object uit verschillende hoeken. Normaal gesproken worden deze twee foto's gemaakt door de camera om het voorwerp heen te verplaatsen of door meerdere camera's te gebruiken. Geen van deze methodes kon door ons worden gebruikt in combinatie met röntgenfotografie, aangezien er maar één röntgenbron beschikbaar was en verplaatsing van de camera resulteert in een verandering van de inwendige oriëntering (onderlinge samenhang van bron en film) van de camera. Dit komt doordat in röntgenfotogrammetrie de röntgenbron en de film niet met elkaar zijn verbonden en bij een beweging van de camera de bron en de film nooit op precies dezelfde manier kunnen worden verplaatst. Elke verandering in de configuratie van de camera vermindert de precisie van de berekeningen. In samenwerking met de Technische Universiteit Delft is een nieuwe methode ontwikkeld om deze problemen op te lossen. Er is één röntgenbron gebruikt en in plaats van de camera te bewegen, is het te fotograferen object verplaatst binnen het bereik van de röntgenbron en gefotografeerd vanuit zes verschillende hoeken. Met deze röntgenfoto's zijn coördinaten van markers bepaald met een nauwkeurigheid van 0.12mm.

In hoofdstuk vijf is beschreven hoe de röntgen stereo-fotogrammetrie methode is gebruikt om de verplaatsing van diverse botelementen in het kaakapparaat, inclusief het PPC, te bepalen tijdens het openen van de bovensnavel. Dit is gedaan voor vijf verschillende soorten, drie Palaeognathae, een prokinetische en een rhynchokinetische neognathe soort. Er is aangetoond dat Bock's kwalitatieve beschrijving (1964) van de beweging van het PPC tijdens het openen van de bovensnavel juist was en dat er geen grote verschillen zijn in het bewegingspatroon van het PPC tussen soorten met verschillende types van kinese.

In de driedimensionale kinematische analyse is de elevatie van de bovensnavel opgelegd door externe krachten. Aangezien iedere structuur zal buigen indien er voldoende kracht op wordt uitgeoefend, is onderzocht of de morfologie van de snavel van de Palaeognathae aangepast is voor rhynchokinese. Voor de analyse van de morfologie van de snavel is gebruik gemaakt van de snavelkenmerken zoals gevonden in de schedel van de distaal rhynchokinetische Kanoetstrandloper (*Calidris canutus*). De morfologische kenmerken die aanwezig moeten zijn om rhynchokinese mogelijk te maken, zijn: duidelijke buigzones in de ventrale (onderste) en dorsale (bovenste) botelementen van de bovensnavel, ontkoppeling van de beweging in de ventrale en dorsale elementen van de bovensnavel en voldoende spierkracht om beweging van de bovensnavel mogelijk te maken. Daarnaast is onderzocht of de kracht van de pterygoid-spier inderdaad bijdraagt aan de totale bijtkracht, zoals voorgesteld door Zweers *et al.* (1997), voor het voorouderlijk palaeognathe voedselopname systeem. De ontkoppeling van de beweging in de dorsale en ventrale botelementen was duidelijk waarneembaar. Er zijn echter geen duidelijke buigzones aangetoond in de botelementen van de bovensnavel. Enkele ietwat dunnere zones zijn waarneembaar in de botelementen van de bovensnavel, maar de positie van deze zones verschilt van de positie waar normaal gesproken buiging wordt waargenomen. De analyse van de spierkrachten laat zien dat de kracht van de *Musculus protractor pterygoidei et quadrati* groot genoeg is om de bovensnavel te openen. De analyse toonde ook aan dat de configuratie van de spieren en de schedel zodanig is dat het resulteert in een zeer lage totale bijtkracht. Een opmerkelijk kenmerk van de palaeognathe schedel is de aanwezigheid van een grote *Processus basipterygoideus*. Dit element kan in combinatie met de grote *Musculus pterygoideus* functioneren als een stabiliserings-mechanisme, wat er voor zorg draagt dat de bovensnavel niet wordt ge-eleveerd of gedepresseerd onder invloed van externe krachten. Grote externe krachten die de bovensnavel opentrekken kunnen vóórkomen tijdens het grazen van planten, wanneer bladeren van de moederplant worden getrokken. Deze resultaten wijzen erop dat de snavel van de Palaeognathae niet ge-adapteerd is voor actieve rhynchokinese, maar dat de beweging in de bovensnavel plaats vindt als gevolg van de natuurlijke buiging die het bot zelf heeft.

In hoofdstuk zeven is het voedselopname gedrag van de Palaeognathae bestudeerd. De transportfase van het voedselopname patroon is een eenvoudige ‘Catch & Throw’ beweging. Drink gedrag is basaal en bestaat uit een scheppende beweging gevolgd door een tip-up fase met lage amplitude. Craniale kinese is beperkt en vindt alleen plaats tijdens maximale opening van de snavel en is altijd naar boven gericht (elevatie). Er is gevonden dat buiging optreedt over de gehele lengte van de bovensnavel, met name vlakbij het puntje van de snavel. De ‘oppak’-fase en de transport fases in het voedselopname gedrag lijken sterk op de fases zoals deze worden gevonden bij Neognathae en er is geen indicatie dat de Palaeognathae onderhevig zijn aan andere selectieve krachten ten gevolgen van voedsel opname dan de Neognathae.

In hoofdstuk acht wordt de eerder genoemde neotenie-hypothese getest. Er is een numerieke methode gebruikt om het PPC van volwassen Palaeognathae te vergelijken met diverse ontwikkelingsstadia van een neognathe vogel. Dit toont aan dat er grote verschillen bestaan tussen de morfologie van volwassen Palaeognathae en de ontwikkelingsstadia van een neognathe vogel, wat er op wijst dat de neotenie-hypothese onjuist is.

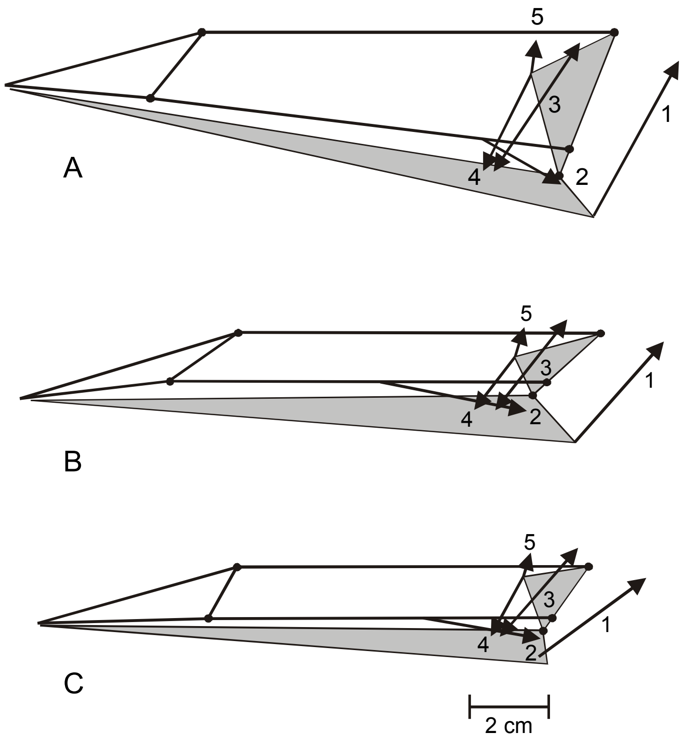
Evolutie tijdens het Krijt

De resultaten van dit proefschrift zullen worden gebruikt om de hypothese van Zweers *et al.* (1997; zie ook Zweers & Vanden Berge, 1997; voor nederlands zie ook Zweers, 1999) omtrent de evolutie van de vogels te testen. Er wordt vanuit gegaan dat de oorsprong van de vogels ligt in de Coelosauria en dat vogels waarschijnlijk nauw verwant zijn aan de Dromaeosauria (Padian & Chiappe, 1988). De eerste stap tijdens de evolutie in de richting van het moderne voedselopname-mechanisme van vogels is de fenestratie (het ontstaan van gaten/vensters) van de schedel en de reductie van het aantal botelementen in het laterale (zijkant) vlak van de schedel zoals beschreven door Zweers *et al.* (1997). Deze reductie en fenestratie resulteerde in een flexibele schedel zonder mechanisme voor craniale kinese. Het bewegelijk worden van het palatinum wordt beschouwd als de volgende stap in de evolutie. Dit werd op drie verschillende wijzen gerealiseerd, wat resulteerde in een drievoudige splitsing in de evolutie. De drie lijnen worden beschreven als een niet-kinetische lijn, een pre-rhynchokinetische lijn (vanaf nu palaeognathe lijn genoemd) en een pre-prokinetische lijn (vanaf nu neognathe lijn genoemd). Aangezien moderne vogels alleen in de laatste twee lijnen worden gevonden, zullen we onze aandacht alleen op deze twee richten. De splitising in drieën is gebaseerd op de reductie van het pterygoid en de mogelijkheid om krachten over te brengen tussen het quadratum en de bovensnavel. Het belangrijkste verschil tussen de palaeognathe lijn en de neognathe lijn van Zweers *et al.* (1997) is de aanwezigheid van een vomerale-maxillaire verbinding. Andere kenmerken die verschillen in de twee lijnen zijn aanwezig in het pterygoid, maar deze vallen niet volledig samen met de palaeognathe en de neognathe lijn. In beide lijnen zijn twee pterygoid-delen aanwezig tijdens ontwikkeling, waarvan het voorste deel of met het palatinum fuseert of volledig verdwijnt. Opvallend is dat de veronderstelde verdeling leidt tot de aanwezigheid van a-kinetische soorten in alle drie de lijnen en dat de mogelijkheid tot kinese zich in elke lijn afzonderlijk ontwikkelt. Onze analyse toont aan dat in elk van de veronderstelde kinetische lijnen zowel de rol en de positie van de botelementen van het PPC (behalve het vomer) als het bewegingspatroon van het PPC vrijwel identiek zijn. Dit duidt erop dat het loskomen van het palatinum eerder in evolutie heeft plaatsgevonden, resulterend in een kinetische en een akinetische lijn. Met deze aanpassing is het transformatie schema van Zweers *et al.* (1997) gelijk aan de klassieke veronderstelling dat de Palaeognathae basaal zijn binnen moderne vogels en de zustergroep (meest nauw-verwante groep) van de Neognathae (Cracraft, 1986; Sibley & Ahlquist, 1990). Gebaseerd op de gegevens van Cooper & Penny (1997) is de kinetische lijn ontstaan tijdens het Krijt, bijna direct gevolgd door een splitsing in de Neognathae en de Palaeognathae.

De overgang van het Krijt naar het Tertiair

De volgende stap in de evolutie van de vogels is de overleving van overgang van het Krijt-tijdperk naar het Tertiar-tijdperk. Deze overgang, 65 miljoen jaar geleden, wordt de K-T grens genoemd en is de periode waarin alle dinosauriërs uitstierven. Een belangrijke ecologische ramp tijdens deze periode was dat er voedselschaarste optrad. Zweers *et al.* (1997) beschrijven voor de Palaeognathe-lijn de mogelijke overgebleven voedselbronnen als zijnde stug en taai plantaardig voedsel. Ze veronderstellen dat voor het eten van dit voedsel de volgende adaptaties noodzakelijk zijn: grote bijtkrachten, een in hoge mate aan te passen rhamphoteca (leerachtige of nagelachtige laag om de snavel heen) en nekwervels die ervoor zorgen dat de nek goed beweegbaar is (hetereocoele wervels). In deze analyse hebben we ons speciaal gericht op de bijtkracht aangezien dit een functionele eis is die direct werkt op het kaak-apparaat. Zweers *et al.* postuleren dat een hoge bijtkracht mogelijk is doordat de schedel kinetisch en het palatinum beweeglijk is, en door een extra kracht component van de *Musculus pterygoideus* (Fig. 10.4). Er wordt aangenomen dat na het beweeglijk worden van het palatinum, de *Musculus pterygoideus* met een extra, op het palatinum werkende, component bijdraagt aan de bijtkracht van zowel de boven- als ondersnavel.

De aannames van aanzienlijke extra bijtkracht lijken onjuist voor de palaeognathe lijn. Het is

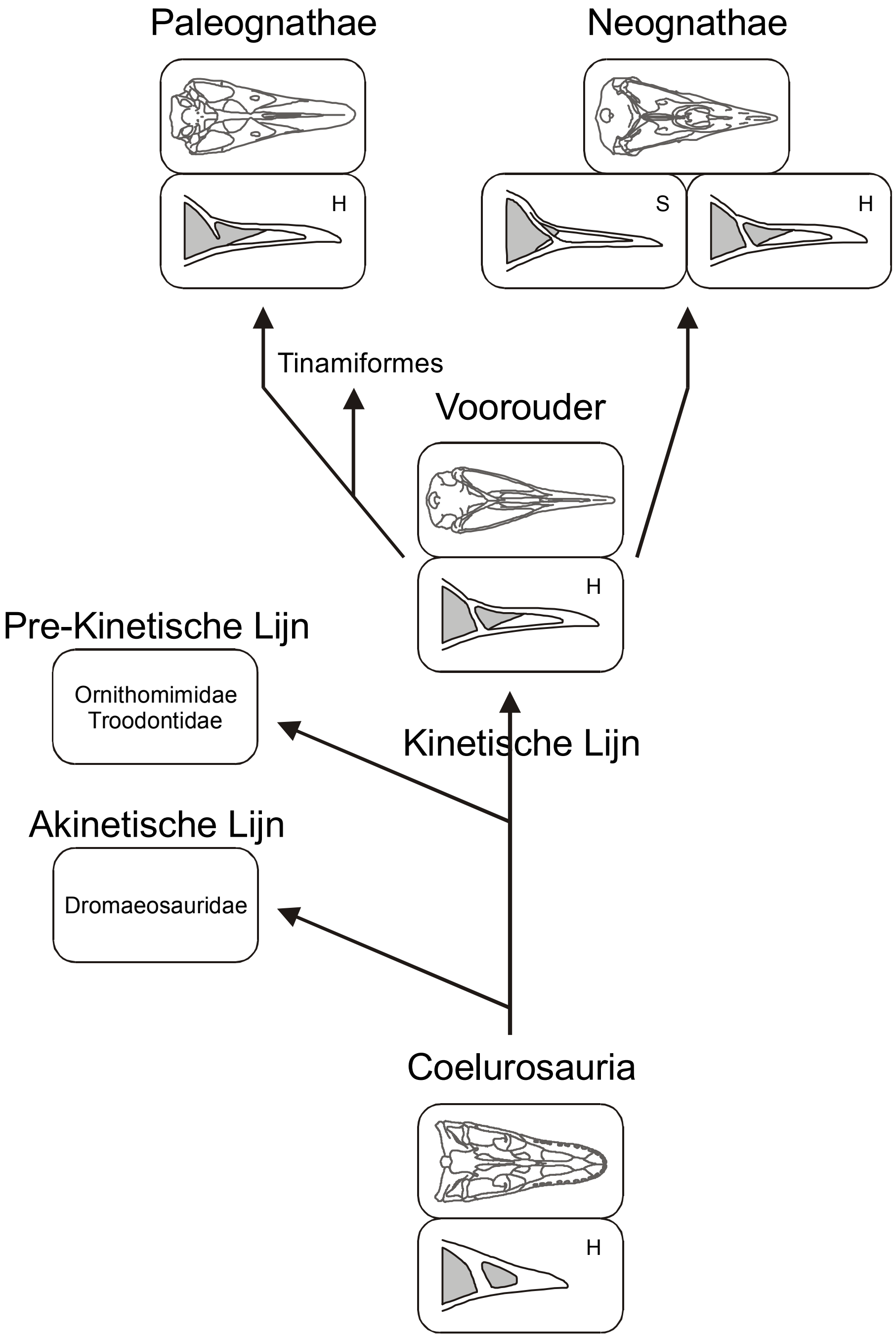


Figuur 10.4. Schematische voorstelling van de schedel van drie soorten Paleognathae. A. Struisvogel (Struthio camelus), B. Emoe (Dromaius novaehollandiae), C. Nandoe (Rhea americana). Pijlen representeren de richting en lengte van de belangrijkste spiergroepen.: 1. Musculus depressor mandibulae, 2. Musculus pterygoideus, 3. Musculus adductor mandibulae externus, 4. Adductor spier complex van het quadratum, 5. Musculus protractor pterygoidei et quadrati. Grijze gebieden representeren het quadratum en de onderkaak. Draaipunten zijn aangegeven met kleine cirkels.

echter mogelijk met het voedselopname gedrag van moderne palaeognathe om op een alternatieve wijze stug en taai plantaardig voedsel te vergaren. De Palaeognathae gebruiken geen grote krachten tijdens de voedselopname (Hoofdstuk 7) maar hebben een mechanisme ontwikkeld dat weerstand biedt tegen elevatie van de bovensnavel als gevolg van grote externe krachten. De algehele morfologie van de schedel laat ook zien dat de noodzakelijke aanpassingen voor rhynchokinese in de bovensnavel ontbreken. Als meest belangrijke punt is aangetoond dat de goed ontwikkelde pterygoid spieren geen significante bijdrage leveren aan de totale bijtkracht (Hoofdstuk 6). We concluderen daarom dat de hypothese van Zweers *et al.* (1997) dat de *Musculus pterygoideus* in combinatie met een bewegelijk palatinum de bijtkracht vergroot, niet juist is voor de Palaeognathae. Zoals terecht opgemerkt door Zweers *et al.* (1997) werkt de *Musculus pterygoideus* zowel op de bovensnavel als de ondersnavel. Als gevolg van de zeer ineffectieve werklijn van de spier op de onderkaak echter, is de bijdrage van de spier aan bovenkaak depressie veel groter dan aan onderkaak elevatie, met als gevolg dat de onderkaak ook wordt gedepresseerd. De depressie van boven- en ondersnavel moet worden gecompenseerd door de activatie van de adductor spieren van de onderkaak. Het is daarom dat, indien de pterygoid spier in grootte toeneemt, de adductor spieren ook moeten toenemen, om zodoende de krachten op beide snavels in evenwicht te houden.

Hoewel de bijtkracht niet toeneemt door de ontkoppeling van het palatinum, is het niet onmogelijk dat de configuratie van het palaeognathe PPC op een andere manier de passage van de K-T grens heeft mogelijk gemaakt. Indien de voorouderlijke Palaeognathae inderdaad leefden van stugge en taaie bladeren tijdens te K-T overgang is het aannemelijk dat zij hierbij dezelfde voedselopname methode gebruikten als de moderne Palaeognathae. Deze vogels verwijderen bladeren van planten, of uit de grond (b.v. grassen), door middel van een naar achter gerichte rukkende beweging met een vertikaal georiënteerde kop (Hoofdstuk 7). Deze beweging resulteert bij stugge planten in grote externe krachten op de bovensnavel, wat kan resulteren in het open gaan van de bovensnavel en dus verlies van grip op het voedsel. De grote *Musculus pterygoideus* kan worden gebruikt om deze externe krachten te compenseren. De aanwezigheid van de *Processus basipterygoideus* voorkomt overcompensatie, resulterend in een depressie van boven- en ondersnavel, doordat deze processus de achterwaartse beweging van het palatinum blokkeert (Hoofdstuk 6).

Het feit dat de bovensnavel moet worden gestabiliseerd door spieractiviteit duidt erop dat de voorouderlijke soorten van de Palaeognathae kinetisch waren, en dat het stabiliserend mechanisme later in de evolutie aan de configuratie is toegevoegd. Een belangrijke vraag blijft echter of de voorouder van de Palaeognathae prokinetisch dan wel rhynchokinetisch was. Zoals eerder vermeld zijn duidelijke buigzones in de bovensnavel afwezig, wat erop duidt dat de voorouder niet rhynchokinetisch was. De ventrale en dorsale botelementen zijn echter wel ontkoppeld in hun beweging, maar door middel van een ander mechanisme dan gevonden wordt in neognathe vogels. De aanwezigheid van een holorhinaal neusgat duidt op een oorsprong van de Palaeognathae in een akinetische dan wel een prokinetische voorouder.



Figuur 10.5. Onderschrift op tegenoverliggende pagina.

Een alternatieve hypothese zou kunnen zijn dat het holorhinale neusgat van de Palaeognathae een gevolg is van neotenie. In dat geval is het mogelijk dat de Palaeognathae zijn ontstaan uit een rhynchokinetische voorouder met een schizorhinaal neusgat. Onze analyse van het PPC laat echter zien dat er geen kenmerken aanwezig zijn die ook worden gevonden in embryonale stadia van een neognathe vogel. Om deze reden wordt de hypothese verworpen dat de morfologie van de palaeognathe schedel is ontstaan door neotenie. Het is echter nog steeds mogelijk dat andere kenmerken zoals het niet-vliegen en de donsachtige veren wel het resultaat zijn van neotenie (de Beer, 1956).

De evolutie van de Palaeognathe vogels

Naar aanleiding van de hierboven beschreven resultaten is een nieuw evolutionair-ontwikkelingstraject voor de Palaeognathae beschreven (Fig. 10.5). De afwijkende morfologie van de Palaeognathae maakt het mogelijk om grote externe krachten op de bovensnavel te weerstaan, die voor kunnen komen tijdens het grazen van stugge plantendelen. Er wordt verondersteld dat deze adaptatie het voor de Palaeognathae heeft mogelijk gemaakt om de K-T grens te passeren. Dit bevestigt de hypothese dat de splitsing tussen de Neognathae en de Palaeognathae plaats heeft gevonden tijdens het Krijt (Cooper & Penny, 1997; Zweers *et al.*, 1997). Het identieke bewegingspatroon van het PPC in neognathe en palaeognathe vogels duidt erop dat de voorouderlijke vogel kinetisch moet zijn geweest met een beweegelijk palatinum. Vanuit deze voorouderlijke soort zijn twee lijnen ontstaan, de palaeognathe en de neognathe lijn. Een belangrijk probleem is het bepalen van de configuratie van de schedel van de vooroudersoort. Aangezien zowel de Palaeognathae als de Neognathae direct uit de voorouder soort zijn onstaan (neotenie speelt geen rol) moeten alle elementen die in één van de twee lijnen aanwezig zijn, ook aanwezig zijn in de vooroudersoort. Om deze reden moet de vooroudersoort van alle moderne vogels zowel een *Vomer* als een *Processus basipterygoideus* bezitten. Aangezien een holorhinaal neusgat zowel in de Palaeognathae als de Neognathae

Figuur 10.5. (op tegenoverliggende pagina) Aangepaste evolutionaire geschiedenis van de Palaeognathae gebaseerd op de hypothese van Zweers et al. (1997). Binnen de Coelurosauria ontstaat eerst een akinetische lijn, waarin de Dromaeosauridea worden geplaatst. De andere lijn heeft gereduceerde craniale botelementen en wordt als pre-kinetisch beschouwd. De laatst genoemde lijn splitst in een kinetische lijn (Avialae) en een pre-kinetische lijn gebaseerd op een beweegelijk pterygoid-palatinum complex. In de pre-kinetishe lijn zitten de Ornithomimidae en de Troodontidae. Het voorouderlijk ontwerp binnen de kinetische lijn heeft een beweeglijk pterygoid-palatinum complex, een holorhinale configuratie van het neusgat en is (pre-) prokinetisch. Door verschillen in de manier van foerageren heeft een splitsing tussen de Palaeognathae en Neognathae plaatsgevonden. Deze splitsing heeft waarschijnlijk plaatsgevonden vóór de K-T grens. Binnen de Palaeognathe lijn zijn de Tinamous waarschijnlijk de meest basale groep.
H=holorhinale configuratie van het neusgat, S=schizorhinale configuratie van het neusgat.

aanwezig is, wordt verondersteld dat de vooroudersoort eveneens een holorhinaal neusgat bezat en waarschijnlijk (pre-) prokinetisch was. De algemene configuratie van de schedel van de voorouderlijke vogelsoort kan daarom sterk geleken hebben op de configuratie zoals deze te vinden is in hedendaagse Tinamiformes. Binnen de morfologie van de schedel van de Tinamiformes zijn alle elementen van het Palaeognathe PPC aanwezig en is het neusgat holorhinaal met een ononderbroken lateraal botelement. Een diversificatie in het voedselopname gedrag heeft mogelijk geresulteerd in de splitsing tussen de Neognathae en de Palaeognathae. De palaeognathe lijn ging over op het fourageren op voedseltypen waarvoor grote krachten noodzakelijk zijn om ze te vergaren en op te eten. Hoewel het onaannemelijk is dat de Palaeognathae de enige vogelgroep vormen die op deze voedseltypen fourageerde, wordt aangenomen dat de specifieke problemen van het foerageren op deze voedseltype in de beide lijnen verschillend zijn opgelost. In de neognathe lijn werden de taaie, stugge voedseldelen gesneden door middel van scherpe randen op de rhamphotheca, die niet ontwikkeld zijn binnen de Palaeognathae. Binnen de Palaeognathae wordt voor het verzamelen van het voedsel gebruik gemaakt van krachten uit de nek, en zonder mogelijkheid om het voedsel te verkleinen ontwikkelde zich een voedselopname gedrag wat geoptimaliseerd is voor het intra-oraal transport van groot voedsel. Het gebruik van nekkrachten resulteerde in een secundaire versterking van de schedelmorfologie om de snavel te stabiliseren. Dit resulteerde in een sterk, verstevigd PPC. Het voedselopname gedrag, waarbij de nek de krachten genereert voor het verkrijgen van voedsel, kan geresulteerd hebben in een toename van de grootte van de dieren, waardoor nog grotere krachten konden worden gegenereerd.

De hypothese van Zweers *et al.* (1997) dat de Palaeognathae voldoende waren geadapteerd om de K-T grens te passeren wordt onderschreven. Daarnaast veronderstel ik dat de Palaeognathae vóór de K-T grens zijn ontstaan uit een (pre-) prokinetische voorouder. Gebaseerd op de aanwezigheid van de grote *Processus basipterygoideus* en het grote *Vomer* moet het PPC van de Palaeognathae sterk lijken op het PPC van de vooroudersoort. Ik concludeer dat de Palaeognathae de eerst ontstane groep zijn binnen de moderne vogels en de zustergroep van alle andere levende vogels.

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CURRICULUM VITAE

Sander Wouter Sebastiaan Gussekloo werd op 3 september 1971 geboren te Wassenaar. Zijn opleiding volgde hij aan het Erasmus College te Zoetermeer, waar hij in 1989 zijn VWO-diploma behaalde. In datzelfde jaar begon hij met de studie Informatica aan de Universiteit Leiden. Na het eerste jaar besloot hij dat hij genoeg abstracte wetenschap had gezien en ging in 1990 Biologie studeren te Leiden.

In zijn doctoraalfase liep hij hoofdstage bij de sectie Evolutiebiologie, waar hij onderzoek deed aan de thermo-regulatie van het tweestipslieveheersbeestje (*Adalia bipunctata*). De stage resulteerde een artikel wat samen met Peter de Jong en Paul Brakefield werd gepubliceerd. Zijn tweede stage liep hij bij de sectie Diersystematiek, waar onderzoek werd gedaan aan landslakken van Maleisië. Hiervoor heeft hij 2 maanden veldonderzoek gedaan op het schiereiland van Maleisië.

Op 29 augustus 1995 behaalde hij de titel doctorandus in de biologie en studeerde *Cum Laude* af, waarna hij op 1 februari 1996 kon beginnen als eerste, en enige, Beurspromovendus van het EEW bij de sectie Evolutionaire Morfologie van de Universiteit Leiden. Zijn onderzoek was voornamelijk gericht op de evolutie van Palaeognathen, waarbij voornamelijk gekeken werd naar de evolutie en functionele morfologie van het kaakapparaat van deze vogels. Naast het onderzoek werd hij tevens aangesteld als docent aan dezelfde universiteit en werkte mee aan eerste-jaars practica, tweede-jaars cursussen en seminaria. Daarnaast werd een aantal stage-studenten begeleid. Ongeveer halverwege de aanstelling werd de status van Beurspromovendus omgezet en werd ook hij officieel AIO (Assistent in Opleiding). Na vier jaar onderzoek en deelname aan diverse internationale congressen hoopt hij op 3 mei 2000 te promoveren aan de Universiteit Leiden. Het promotie onderzoek werd afgerond binnen de periode van vier jaar waarvoor hij was aangesteld.

Op 1 februari 2000 is hij begonnen bij de TU te Delft als Post-Doc, waar hij onderzoek zal doen binnen het DIPEX (Development of ImProved Endoprotheses for the upper eXtrimities) project. Het onderzoek zal gericht zijn op het analyseren en verbeteren van de operatiemethodes bij het plaatsen van schouder- en elleboogprotheses.

Publicaties

de Jong, P.W., S.W.S. Gussekloo & P.M. Brakefield 1996. Differences in thermal balance, body temperature and activity between non-melanic and melanic two-spot ladybird beetles *(Adalia bipunctata)* under controlled conditions. *Journal of Experimental Biology* **199**(12): 2655-2666.

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Een trek van onbeschrijfelijke opluchting verspreidde zich over heer Bommels
trekken toen al het weten uit zijn schedel verdween

Marten Toonder, De Weetmuts (8445)