

Structural tissue organization in the beak of Java and Darwin's finches

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Abstract

Birds are well known for occupying diverse feeding niches, and for having evolved diverse beak morphologies associated with dietary specialization. Birds that feed on hard seeds typically possess beaks that are both deep and wide, presumably because of selection for fracture avoidance, as suggested by prior studies. It follows then that birds that eat seeds of different size and hardness should vary in one or more aspects of beak morphology, including the histological organization of the rhamphotheca, the cellular interface that binds the rhamphotheca to the bone, and the organization of trabeculae in the beak. To explore this expectation we here investigate tissue organization in the rhamphotheca of the Java finch, a large granivorous bird, and describe interspecific differences in the trabecular organization of the beak across 11 species of Darwin's finches. We identify specializations in multiple layers of the horny beak, with the dermis anchored to the bone by Sharpey's fibers in those regions that are subjected to high stresses during biting. Moreover, the rhamphotheca is characterized by a tight dermo-epidermal junction through interdigitations of these two tissues. Herbst corpuscles are observed in high density in the dermis of the lateral aspect of the beak as observed in other birds. Finally, the trabecular organization of the beak in Darwin's finches appears most variable in regions involved most in food manipulation, with the density of trabeculae in the beak generally mirroring loading regimes imposed by different feeding habits and beak use in this clade.

Key words: beak anatomy; birds; Darwin's finches; Herbst corpuscles; histology; *Padda oryzivora*; trabecular structure and organization.

Introduction

Birds are an ecologically diverse and species-rich group of terrestrial vertebrates that have radiated extensively into a variety of habitats and trophic niches (Mayaud, 1950; McLelland, 1979; Raikow & Bledsoe, 2000). Bird beaks are equally diverse, and in many species the diversity in beak size and shape appears to be correlated with feeding habits

(Newton & Gadow, 1896; Bowman, 1961; Pimm & Pimm, 1982; Grant, 1999). Amongst birds, specializations for feeding on hard seeds are common, and many species that feed on hard seeds typically have beaks that are both deep and broad, suggesting that the shape of the beak plays an important role in allowing birds to crack hard seeds. Yet, the ability to crack hard seeds depends primarily on birds' ability to generate high bite forces, via jaw muscles. Consequently, the beak can be thought of as a structure shaped by selection for fracture avoidance as suggested by studies on the mechanical resistance of the beak to loading (Herrel et al. 2005b, 2010; Soons et al. 2010, 2012a,b). Indeed, the evolution of high bite force capacity has gone hand in hand with the evolution of large beak size independently in many radiations of birds specialized in the cracking of hard seeds (Thomson, 1923; Mayaud & Grassé, 1950; van der Meij

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& Bout, 2004; Herrel et al. 2005a,b). Yet, other selective pressures also act on the beak (e.g. Herrel et al. 2009), and the final structure and shape likely reflect a compromise between all of the selective pressures involved.

One of the best studied examples of an adaptive radiation in feeding form and function is Darwin's finches of the Galápagos and Cocos Islands. Darwin's finches are traditionally classified into three functional groups according to their feeding habits and beak morphology (Bowman, 1961; Grant, 1986): first, the crushers with deep, broad beaks (ground finches: *Geospiza magnirostris*, *G. fortis*, *G. fuliginosa*, *G. conirostris*); second, the probers with long, narrow beaks (*Certhidea olivacea*, *Pinaroloxias inornata*, *Cactospiza pallida*, *Ca. heliobates*, *Geospiza scandens*); and third, the tip-biters with curved upper and lower beaks (tree finches: *Camarhynchus parvulus*, *C. pauper*, *C. psittacula*). *Geospiza difficilis* and *Platyspiza crassirostris* are more difficult to assign to any one of these groups. *Geospiza difficilis* has a beak that is deep at its base but is also fairly straight, and thus falls between the first and second groups. *Platyspiza crassirostris* combines characteristics from the second and third groups, and uses the entire length of its beak for biting (Bowman, 1961). Surprisingly, and despite the fact that these finches are some of the best studied cases of adaptive differentiation of morphology in relation to diet, virtually nothing is known about fine-scale morphological adaptations and functional specializations associated with the evolution of their ability to crack hard seeds (but see Bowman, 1961; Herrel et al. 2005a,b, 2009, 2010; Soons et al. 2010). The protected status and restrictions associated with obtaining living or even fresh, dead specimens of Darwin's finches have complicated functional morphological studies.

To explore the hypothesis that beak shape may have evolved in order to avoid fracture in seed-eating birds (Bowman, 1961; Herrel et al. 2005a,b; Soons et al. 2010), researchers have used mechanical models, including finite element models, to predict how different beak morphologies in seed-eating birds should be able to withstand reaction forces generated on the beak during biting (Soons et al. 2010; Rayfield, 2011). Although these models offer a good first approximation of how beaks may respond functionally to loads imposed by seed-crushing or pecking, current models are hampered by two major limitations: a lack of data on how the keratinous rhamphotheca (horny beak) contributes to beak strength (but see Soons et al. 2012a,b); and a lack of data concerning the organization of the bony beak's internal trabecular structure. Realistic models, representative of actual feeding behavior *in vivo*, will require attention to the rhamphotheca, the cellular interface between the horny and bony beak, and the organization of the trabeculae. Moreover, striking variation in the structure and dimensions of the rhamphotheca relative to the underlying bone can be observed in different species of seed-cracking birds (personal observation), consistent with a potentially

important role of the keratinous rhamphotheca in stress dissipation during biting (see also Soons et al. 2012b).

Yet, the histological organization of the rhamphotheca, the bony beak and the cellular interface between these two, remains, to our knowledge, poorly known (Lucas & Stettenheim, 1972; Stettenheim, 1972; Spearman, 1973; Stettenheim, 2000; Van Hemert et al. 2012). These studies show that the rhamphotheca is a thick, modified integument that covers the underlying bone. It is hard and heavily cornified in most birds. The epidermis is thick and composed of beta-keratin-producing cells. The dermis, by contrast, is thin and binds the keratinized epidermis to the bone (Spearman, 1973; Stettenheim, 2000). The organization of the trabecular structure of the bony beak has been described for only a few species, including the toucan, the hornbill (Seki et al. 2005) and the crow (Bock, 1966).

Here we explore fine-scale structure and organization of the rhamphotheca, the bony beak and the cellular interface between the two, with particular attention to how this structure and its organization may potentially enable birds to withstand the strains imposed on the beak by seed crushing. We predict distinct differences in tissue organization in those beak regions that endure the highest strains (see Herrel et al. 2010; Soons et al. 2010), and also expect variation in the trabecular organization of the bony beak among the different species of Darwin's finches mirroring variation in how these birds use their beaks (Bowman, 1961). We begin with a detailed description of the histological and structural organization of the rhamphotheca, the bony beak and the cellular interface in the Java finch (*Padda oryzivora*), a passeriform bird that has been suggested as a suitable model for understanding adaptations to seed cracking (Genbrugge et al. 2011). We then describe, in the Java finch as well as Darwin's finches, the variation in the trabecular structure of the bony beak.

Materials and methods

Specimens

Eight specimens of the Java finch (*Padda oryzivora*) were used in this study. The *Padda oryzivora* specimens were obtained from commercial suppliers and killed by a veterinarian of the Faculty of Veterinary Medicine at Ghent University. Six specimens were preserved in a 10% aqueous formaldehyde solution for 4 weeks. One specimen was preserved in Bouin fixative for 4 weeks. Small parts of the beak of the eighth specimen were transferred to a Karnovsky medium (2% paraformaldehyde, 2.5% glutaraldehyde and 0.5% CaCl₂ in 0.134 M sodium cacodylate buffer) immediately after killing the specimen. These latter samples were stored in the refrigerator (4 °C) for 1 day.

Data on the trabecular organization of the beak were obtained for 22 specimens of Darwin's finches. Eleven specimens of six species (*Camarhynchus parvulus*: 2; *Certhidea olivacea*: 2; *Geospiza fortis*: 2; *G. fuliginosa*: 2; *G. scandens*: 2; and *Platyspiza crassirostris*: 1) were road-killed birds collected during February–March of 2005 and 2006

on Santa Cruz Island, Galapagos. A stretch of road of approximately 5 km was walked continuously every day between sunrise and 13:00 hours, and all road-killed birds that showed no obvious external damage to the head were collected. Specimens were preserved in a 10% aqueous formaldehyde solution for 24 h, rinsed and transferred to a 70% aqueous ethanol solution. These specimens were collected under a salvage permit from the Galápagos National Park Service. The other 11 Darwin's finch specimens were obtained from the collections of the Museum of Comparative Zoology (Harvard University) and the California Academy of Sciences [*Cactospiza pallida* (MCZ65744 and CAS ORN 86881), *Camarhynchus psittacula* (MCZ65738 and CAS ORN 42348), *Geospiza difficilis* (MCZ39828 and CAS ORN 86586), *G. magnirostris* (MCZ112397 and CAS ORN 86316), *Pinaroloxias inornata* (MCZ157930 and CAS ORN 86959) and *Platyspiza crassirostris* (MCZ134639)].

Histological sections

For all specimens of Java finches, histological cross-sections throughout the beak were made by embedding either parts of the beak, a whole beak, or the head. Several embedding media were tested as sectioning of heavily cornified and keratinized tissues proved to be challenging. The embedding media tested were Technovit 7100, Paraffin, Epon and Spurr. For the first three embedding media, specimens were first rinsed with tap water, decalcified with Osteomoll (Merck Cat. No. 1.01736), rinsed again with tap water, dehydrated in a graded (30, 50, 70 and 96%) ethanol solution and transferred to Technovit 7100 (Heraeus Kulzer Wehrheim, Germany), Paraffin (Merk, 9025) or Epon (Fluka, 45359). The small parts of the beak fixed with Karnovsky medium were rinsed with 0.134 M sodium cacodylate buffer for 8 h, decalcified with Osteomoll and rinsed again with the 0.134 M sodium cacodylate buffer. Post-fixation took place overnight in reduced osmium, a mixture of 1 mL OsO₄ (4%), 3 mL Na cacodylate (0.134 M) and 66 mg K₃Fe(CN)₆. After rinsing with double-distilled water, the parts were dehydrated in 50, 70, 96% and absolute ethanol, to which CuSO₄ bars were added to remove any remaining water. The specimens were then transferred to Spurr (no 1969).

Histological sections of the specimens embedded in Technovit 7100 were made on a POLY CUT Leica SM2500 microtome equipped with a wolfram carbide knife. The Paraffin-, Epon- and Spurr-embedded specimens were cut with a MICROM HM360 microtome equipped with disposable microtome blades (Superlab; Paraffin) and diamond knives (Epon and Spurr). All sections were cut at a thickness of 2 µm, stained with toluidin blue, mounted with a xylene-based mounting medium and covered.

The histological sections were examined using an Olympus SZX9 stereomicroscope, on which a ColorView 8 digital camera was mounted.

CT-scanning and 3D-reconstruction

All salvaged road-killed specimens were scanned at the UGCT scanning facility at Ghent University (<http://www.ugct.ugent.be>). Reconstruction of the tomographic projection data was done using the in-house developed Octopus-package (Vlassenbroeck et al. 2007). The specimens obtained from the Museum of Comparative Zoology were scanned at the Harvard CNS facility. Reconstruction of the tomographic projections was done using CTPro (Metris). Voxel sizes ranged from 25.26 µm for the smaller species to 45.75 µm for *Geospiza magnirostris*.

The CT-data were loaded into Amira 5.2.2 (64-bit version; Computer Systems Mercury) where the data were first reoriented along the x-, y- and z-axes so that all specimens are oriented along the same axes. The bony beak structures were then segmented semi-automatically based on gray-scale values of the voxels, with manual corrections to remove noise. Surface and volume rendering were also performed in Amira 5.2.2. The trabecular organization was described for the posterior third of the beak anterior to the nares in all specimens.

Results

Epidermis

The epidermis is a thick, stratified squamous epithelium made up of multiple layers of cells. Its thickness varies depending on the location on the beak. Three regions can be distinguished: the dorsal and lateral surface; the lateral sharp edge (tomial edge) of the beak; and the horny pal-ate.

The epidermis at the dorsal and lateral surface has more or less the same organization, comprising a stratum basale, stratum spinosum, stratum transitivum and stratum corneum (Fig. 1A–F). The stratum basale consists of a single layer of columnar cells, between which interdigitations (Fig. 1A, D, F) of the dermis can be found. Several cells show projections into the dermis. The stratum spinosum (Fig. 1A, D, E) consists of four–six layers of cells that are characterized by the clearly visible spinae (Fig. 1E) of adjacent cells. The subsequent stratum transitivum (Fig. 1A–D) consists of multiple cell layers with cells that are getting flatter as they move towards the outer surface. The outer layer of the epidermis is the keratinous stratum corneum (Fig. 1A–D) with very flat and dead cells without a nucleus. They lie next to and on top of each other like thin filaments that have been compressed (Fig. 1B). This stratum corneum is thicker than the other three layers together and broadens towards the tomial edge (Fig. 1G). Moving from the caudal part of the beak to its tip, the living part largely maintains its thickness, although the cells of the stratum spinosum and transitivum are somewhat more compressed at the level of the nares. The cells of the stratum transitivum and spinosum are orientated parallel with the surface of the rhamphotheca (Fig. 1K) instead of in line with the cells of the stratum basale. This becomes clearer towards the tip of the beak. The lifeless stratum corneum is relatively thicker at the tip of the beak. The tip of the outer beak is made up of only cells of the stratum corneum.

The tomial edges (Fig. 1G) are characterized by a very thick epidermal layer, which consists almost completely of keratinized and cornified cells of the stratum corneum. The cells of the stratum corneum are round except at the dorsal and lateral side of the tomial edge. At those points the cells change their shape and become flat cells typical of the lateral aspect of the beak (Fig. 1I). In the center of the stratum corneum of the tomial edge, round packets of uncornified

cells can be found (Figs 1G,H and 2G–I). These are probably cells from the stratum basale that surround projections of the dermis into the tomial edge.

The epidermis of the horny palate (Fig. 1J) is very thick (two–three times the thickness of the lateral and

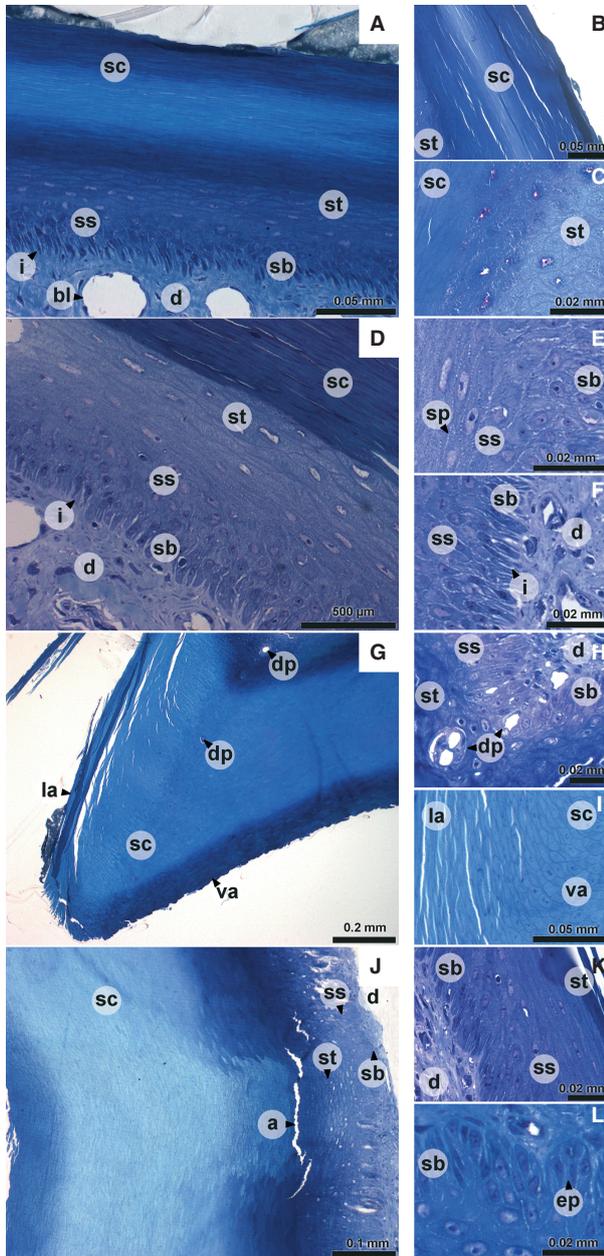


Fig. 1 Digital pictures of histological sections of the epidermis of the upper beak of *Padda oryzivora*. (A) dorsal aspect of the upper beak; (B, C, E, F, K) detail of lateral aspect of the upper beak; (D) lateral aspect of the upper beak; (G) tomial edge; (H, I) detail of tomial edge; (J) ventral aspect of the upper beak; (L) detail of ventral aspect of the upper beak. a, artifact; bl, blood vessel; d, dermis; dp, dermal projection in the epidermis; ep, epidermal papilla; i, interdigitations; la, lateral aspect of the upper beak; sb, stratum basale; sc, stratum corneum; sp, spinae; ss, stratum spinosum; st, stratum transitivum; va, ventral aspect of the upper beak.

dorsal surface) and characterized by a well-developed relief with ridges and grooves. The strata basale, spinosum and transitivum in this region have the same thickness as in the lateral and dorsal aspect of the beak. However, the thickness of the stratum corneum of the horny palate is much greater. The cells of the stratum basale are broader than those at the lateral aspect of the beak and are organized in epidermal papillae (Fig. 1L). These dermal papillae are larger at both the base and the tip of the beak. Sometimes a lamina basalis is distinguishable as a darker line between the epidermal cells and the dermis. The appearance of the cells of the stratum corneum changes from the base of the beak to its tip, with cells at the caudal-most point of the beak being very flat. Just anterior the nares, the cells of the stratum corneum are rounder, even at the surface. Going more rostrally, the cells again become more flattened. The grooves and ridges are visible only in the stratum corneum. At some places the organization of the horny palate is also visible in the stratum transitivum as an accumulation of cells at the ridges or a decrease in the number of cells in the grooves (Fig. 1J).

Dermis

The dermis consists of a single layer of dense irregular connective tissue in which blood vessels, mechanoreceptors and nerves can be found. The presence of these elements depends on the region of the beak dermis considered. Four regions can be distinguished: the dorsal aspect, the lateral aspect, the tomial edge and the palate. At the dorsal face of the upper beak (Fig. 2A), the dermis contains numerous large blood vessels (Fig. 2A,B), often running through the entire thickness of the dermis. The dermis in this area also contains a few small nerve bundles (Fig. 2A). The large blood vessels become much smaller when moving more laterally through the beak. Additionally, when going from caudal to rostral the blood vessels become smaller and less numerous. The blood vessels found at the lateral aspect of the beak are generally smaller, less abundant and lie on the epidermic side (Fig. 2C,D). The dermis of the lateral aspect of the beak (Fig. 2D) is characterized by mechanoreceptors together with a larger number of nerve bundles, which have a larger diameter than those of the dorsal aspect of the beak. The mechanoreceptors are Herbst corpuscles that are visible as large corpuscles with a central axon surrounded by nuclei of Schwann cells and a concentric network of collagen fibers (Fig. 2D,F). The nerve bundles and Herbst corpuscles are situated close to the bone (Fig. 2D,C). The Herbst corpuscles can be found at the entire lateral aspect of the beak (see also Van Hemert et al. 2012), but are absent from the tip in front of the bone. At the level of the tomial edge, projections of the dermis run into the epidermis (Figs 1G,H and 2G–I). Possibly, these projections are

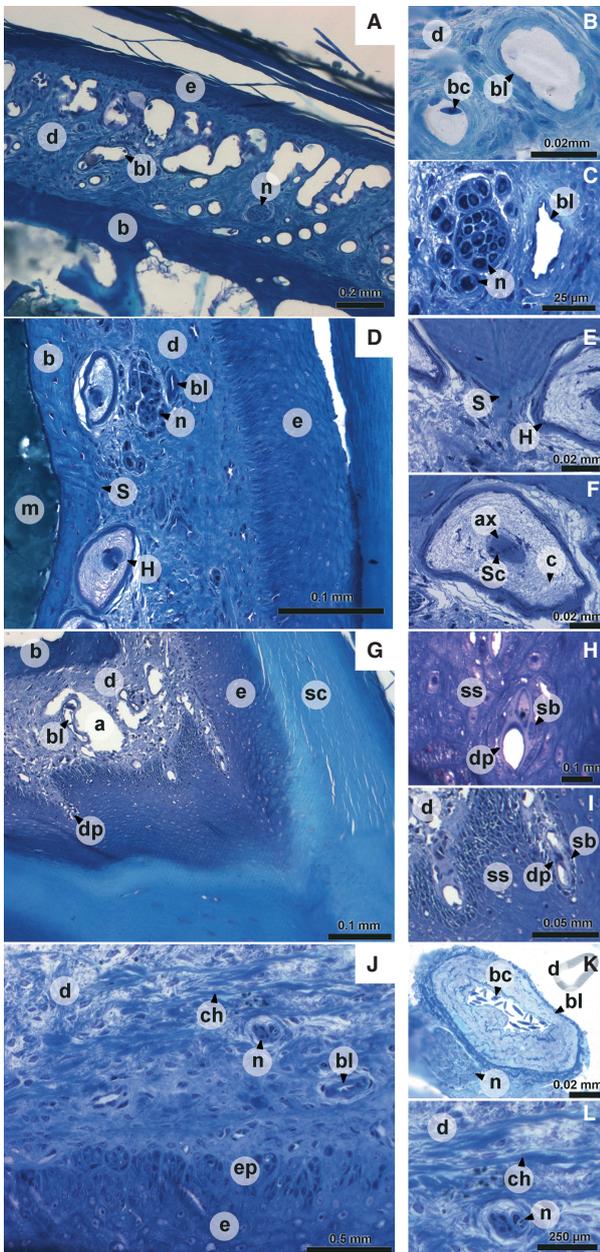


Fig. 2 Digital pictures of histological sections of the dermis of the upper beak. (A) dorsal aspect of the upper beak; (B) detail of dorsal aspect of the upper beak; (D) lateral aspect of the upper beak; (C, E, F) detail of lateral aspect of the upper beak; (G) tomial edge; (H, I) detail of tomial edge; (J) ventral aspect of the upper beak; (K, L) detail of ventral aspect of the upper beak. a, artifact; ax, axon; b, bone; bc, blood cell; bl, blood vessel; c, collagen fibers; ch, horizontally oriented collagen bundle; d, dermis; dp, dermal projection in the epidermis; e, epidermis; ep, epidermal papilla; H, Herbst corpuscle; m, bone marrow; n, nerve bundle; S, Sharpey's fiber; sb, stratum basale; sc, stratum corneum; Sc, nucleus of Schwann cell; ss, stratum spinosum.

connected to the small round packets with uncornified cells in the stratum corneum of the tomial edge, described above. However, this cannot be verified based on our histological sections. The dermis of the palate (Fig. 2J) is thinner,

and Herbst corpuscles, nerves and blood vessels are scarce. Only medially, several Herbst corpuscles, large bundles of nerves, and large arteries (Fig. 2K) and veins can be observed.

In addition to the presence of sensory and vascular elements, the dermis of the beak is characterized by distinct, dense bundles of collagen fibers. These bundles are very abundant near the base of the beak (at the level of and just anterior to the nares) on both the lateral and dorsal aspect of the beak. The collagen bundles become less abundant and their thickness diminishes more rostrally in the beak. At the lateral and dorsal aspect of the base of the beak, these bundles lie in close contact with the bone and regularly penetrate it as Sharpey's fibers (Fig. 2D,E). These penetrations are mostly associated with small protrusions of the bone. Most of the bundles are oriented dorsally away from the bone. In the dermis of the palate of the beak, the collagen fibers are thinner and oriented more horizontally (Fig. 2L).

Bone

The praemaxillary bone is situated in the center of the beak. It consists of a thick outer bony shell filled with numerous bony, pillar-shaped trabeculae, in a foam-like structure. On its outer surface, the bone shows small protrusions in which collagen fibers penetrate. These protrusions are mostly found in the region just anterior to the nares and especially on the lateral aspect of the bony beak. The spaces between the trabeculae are occupied by adipose tissue. The trabeculae also surround several canals through the bone in which blood vessels and nerve bundles run.

The organization of the internal trabeculae and their thickness differs from species to species. In *Padda oryzivora* (Fig. 3) the upper beak is filled with numerous, fine trabeculae just anterior to the nares (Fig. 3D). Additionally, the tip of the beak is filled with slender trabeculae (Fig. 3B). In the middle portion of the beak, only the lateral sides of the beak show small trabeculae (Fig. 3C,D). The center is an open space with only a couple of larger and thicker vertically oriented trabeculae present.

Based on the organization and structure of the trabeculae in the upper beak, just anterior to the nares, the Darwin's finches can be divided into four groups largely corresponding to previously defined functional groups (Bowman, 1961; Figs 4 and 5): in the first group a medial zone is observed where trabeculae are concentrated, and comprises the base crushing *Geospiza magnirostris*, *G. fortis*, *G. fuliginosa*; the second group is characterized by two more lateral zones with more intense trabeculation (left and right side of the beak) and a large cavity in the center of the middle third of the beak, and is composed of the species with probing beaks including *Geospiza scandens*, *G. difficilis*, *Pinaroloxias inornata*, *Certhidea olivacea*; the third group is characterized by thin and seemingly unorganized trabeculae with the entire tip of the beak filled by

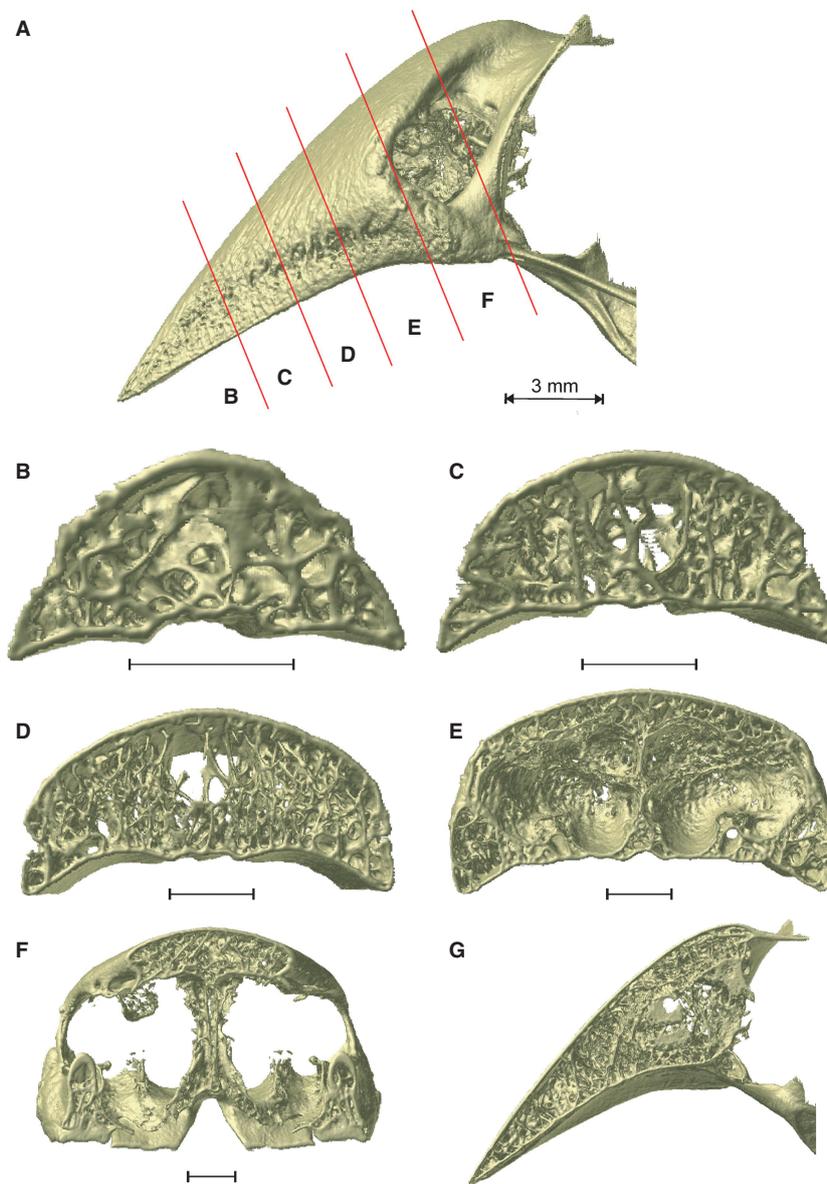


Fig. 3 Organization of the trabeculae inside the upper beak bone of *Padra oryzivora*. (A) Lateral view with the position of the cross-sections indicated by the red lines; (B–F) caudal view of the cross-sections through the upper beak; (G) midsagittal cross-section through the upper beak. Scale bars below the cross-sections represent 1 mm.

small trabeculae (this third group comprises the tip-biting species *Camarhynchus psittacula*, *C. parvulus*, *Cactospiza pallida*); the fourth group represents a mix between the morphologies of Groups I and III, and is composed of the vegetarian finch, *Platyspiza crassirostris*.

For the first group, concentration of trabeculae in this medial zone is broadest and densest in *Geospiza magnirostris*, and occupies almost the entire width of the bony beak. The trabeculae in *Geospiza fortis* tend to fuse with one another, thereby forming a kind of central bony plate. In *Geospiza fuliginosa*, only a few large trabeculae are situated medially in the beak, rendering it the least 'typical' of this group. The other two-thirds of the beak of *Geosp-*

iza fuliginosa are characterized by a central open space. Only in the outermost lateral corners of the beak and the very tip, can trabeculae be found. This central open space is smaller in *Geospiza fortis* and is almost absent in *G. magnirostris*, where nearly the entire beak is filled with trabeculae. Interestingly, in this group the trabeculae within the mediosagittal plane diverge in the region anterior to the fusion of the os palatinum with the os praemaxillare (Fig. 4B).

In the second group, the lateral zones of denser trabeculation, just anterior to the nares, range from being composed of several small trabeculae in *Geospiza scandens* to one thick trabecula on each side as in *Certhidea olivacea*.

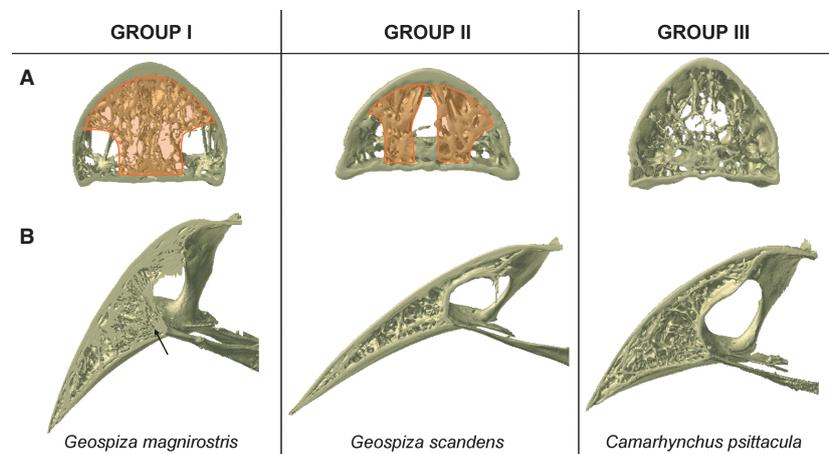


Fig. 4 Organization of the trabeculae into three groups, showing: (A) the region just anterior to the nares (cross-section D in Fig. 3) with an indication of the zones of concentrated trabeculation in orange; (B) the midsagittal plane. Note the diverging trabeculae at the point where the os palatinum fuses with the upper beak in *Geospiza magnirostris*, indicated with an arrow.

The tip of the beak in these species is filled with trabeculae of which the abundance diminishes going from *Geospiza scandens* to *Certhidea olivacea* (in the above-mentioned sequence). Central in the beak a hollow space is situated, which stretches to the dorsal surface of the bony shell, such that only the ventral side and the lateral corners show small trabeculae.

The third group comprises species of which the beaks are completely filled with seemingly unorganized trabeculae just anterior to the nares. The trabeculae are numerous and fine in *Camarhynchus psittacula*, but larger and less numerous in *Cactospiza pallida*. The tips of these beaks are also filled with numerous small trabeculae. The middle third of the beak is hollow centrally, with only small trabeculae on the dorsal side and the lateral corners of the beak being present. In *Cactospiza pallida* this central cavity is largest as it reaches the dorsal side of the beak, although a couple of large trabeculae do cross this space.

The organization of the trabeculae in the beak of *Platyspiza crassirostris*, the sole representative of Group IV, is unique. It resembles that of *Geospiza magnirostris* at the point just anterior to the nares, but resembles that of the *Camarhynchus* species in the organization of the trabeculae in the tip, suggesting that the beak in this species is able to withstand both tip and base loading. Moreover, this illustrates how the trabecular organization across species represents a continuum rather than discrete groups.

Discussion

Beak tissue organization and mechanical stress resistance

Our results accord closely with previously published data (Lucas & Stettenheim, 1972; Sawyer et al. 1986; Bragulla & Homberger, 2009; Van Hemert et al. 2012) in suggesting that the upper beak contains four distinct layers: first, the

dead, strongly keratinized outer layer (stratum corneum); second, the living rest of the epidermis (strata transitivum, stratum spinosum = stratum intermedium in Lucas & Stettenheim, 1972 and Sawyer et al. 1986; and stratum basale); third, the dermis; and fourth, the bone. In *Padda oryzivora*, each layer has specific characteristics that are particularly interesting in the context of potential adaptations to the dissipation of mechanical stress. The flake-like cells of the thick stratum corneum probably form a strong unit as the cytoplasm of these cells is filled with coarse bundles of keratin filaments (consisting of the hard avian β -keratins) and the intercellular matrix is filled with cementing substances (Bloom & Fawcett, 1994; Sawyer et al. 2000; Alibardi, 2009). This organization of hard elements (such as calcium Spearman, 1973; Bragulla & Homberger, 2009 and pigments, Alibardi, 2010) gives the stratum corneum its resistance to abrasion (Bonser & Witter, 1993; Seki et al. 2005). The cells of the underlying cell layers of the strata transitivum, spinosum and basale contain keratin filaments in their cytoplasm, which may allow these cells to withstand mechanical stress, maintain their structural integrity, ensure mechanical resilience and provide protection against variations in hydrostatic pressure (Bragulla & Homberger, 2009). In addition to the strengthening resulting from the specific material properties, at the cellular level, the tissue organization shows similar adaptations. Indeed, on the dorsal and lateral aspect of the beak of *Padda oryzivora* the cells of the stratum basale interdigitate with the dermis. The cells of the epidermis of the horny palate are also connected with the dermis, although here the cells of the stratum basale are organized in epidermal papillae. In this way, the epidermis and dermis form a tight dermo-epidermal junction. Furthermore, the dermis also shows a strong interaction with the bone as large, dense bundles of collagen fibers make direct contact with the bone of the beak. Several bundles penetrate deep into the bone and can be

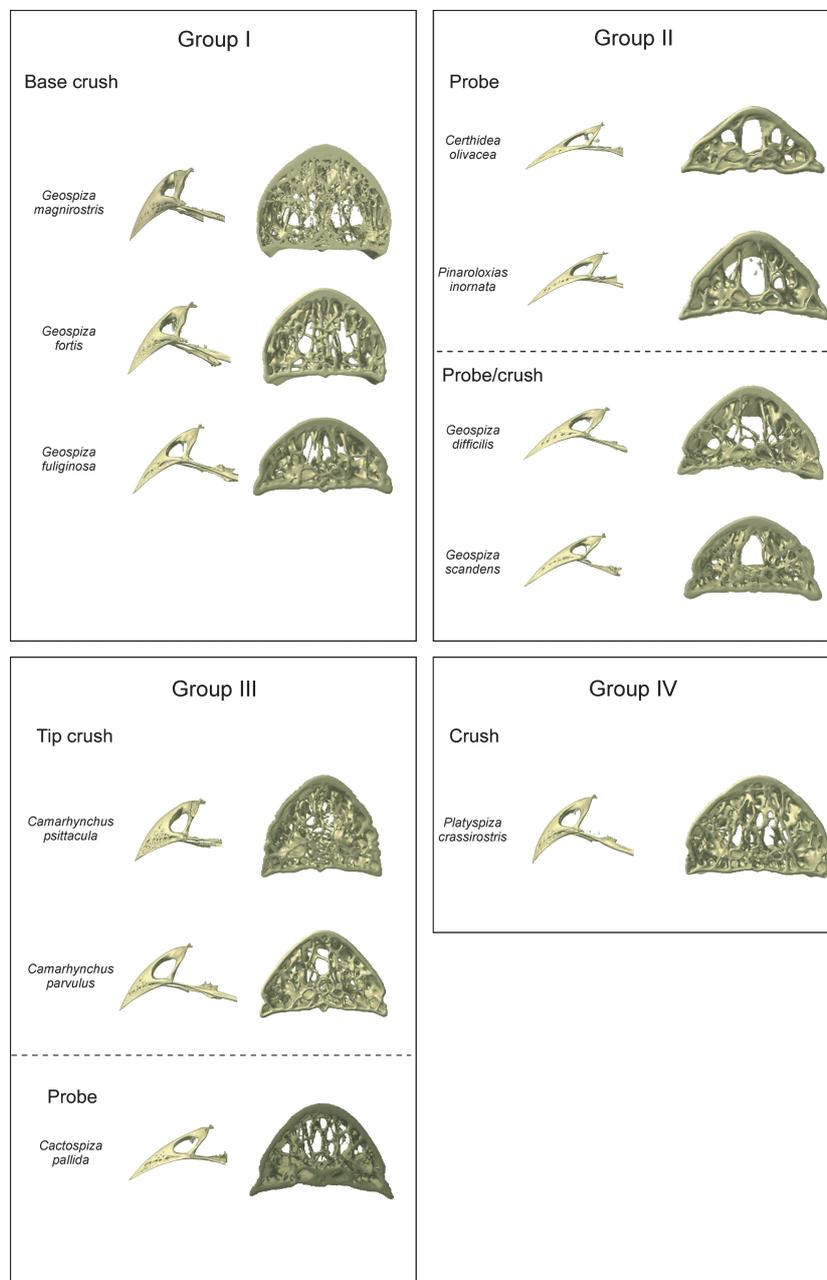


Fig. 5 The beaks of the Darwin’s finches, demonstrating the variation in beak shape and corresponding variation in trabecular organization. The trabecular organization of the region just anterior to the nares is shown (cross-section D in Fig. 3). The classification of the different species into groups is based on the trabecular anatomy. For each group the functional groupings from Bowman (1961) are also represented. Note that although in the section just anterior to the nares *Platyspiza crassirostris* resembles the base-crushing morphology of Group I, near the tip of the beak it resembles the morphology of Group III more.

recognized as Sharpey’s fibers (Francillon-Vieillot et al. 1990), anchoring the dermis to the bone. The abundance of the collagen fibers in the dermis in *Padda oryzivora* likely gives it visco-elastic properties with limited extension capacities, as collagen fibers are only able to compress but not to stretch (Wainwright et al. 1976). The point to which the dermis can be stretched likely depends on the orientation and length of the collagen fibers.

The observed cell orientation in the epidermis of *Padda oryzivora* may also reflect adaptations to differing levels of mechanical stress. As seed-eating birds crack seeds unilaterally, in the groove between the tomial edge and the lateral rim of the palate (van der Meij & Bout, 2006), the rhamphotheca is expected to experience a torque around the bony core, which should in turn impose shear stress at the interface between the bone and the epidermal

cells. More specifically, because the forces induced by cracking a seed are oriented perpendicular to the horny palate at the place where the seed is cracked (van der Meij & Bout, 2006), shear forces can be expected to act at the lateral aspect of the beak. By contrast, the ventral aspect of the beak is expected to have to cope with compressive stress. Our data show that the epidermal cells of the lateral aspect are flat and positioned with their long axis in line with the direction of shear. In contrast, cells of the horny palate are more rounded, making them likely better suited to cope with compressive stresses. Moreover, as mentioned above, the different elements and layers of the beak are firmly anchored to one another. In the dermis at the lateral face of the beak a good anchoring of the rhamphotheca and underlying cellular interface to the bone will be important in resisting the expected shear. It is thus not surprising that there is an abundance of collagen fibers of the dermis at the lateral and dorsal aspect of the beak penetrating into the periosteum and dispersing into the bone. This is in contrast to the horizontally oriented collagen fibers visible at the ventral aspect of the beak, which only occasionally penetrate the periosteum. The finite element models of Soons et al. (2010, 2012a,b) suggest that the stresses in the upper beak induced by the unilateral cracking of a seed at the base of the beak are highest at the dorsal and lateral side of the beak at the level of the nares and lowest at the tip of the beak. These data are in accordance with our observations of the organization of the rhamphotheca, the bony beak and the cellular interface.

The high density of Herbst corpuscles in the dermis is striking. Previously it has been suggested that their function is related to touch, pressure or vibrational stimuli (Mayaud & Grassé, 1950; Portmann, 1950; Stettenheim et al. 1972), or with detecting acceleration components associated with mechanical stimuli (Malinovsky & Pac, 1990). Yet, the exact function of the Herbst corpuscles remains unknown to this date. The frequent occurrence of these corpuscles in the dermis of *Padda oryzivora* and the Black-capped Chickadee (Van Hemert et al. 2012), especially on the lateral aspect of the beak, suggests to us that they may be part of a feedback system that could allow birds to detect stress in the dermis. Such a feedback system has been suggested for mammals, where periodontal receptors encode information on the orientation, magnitude, rate and position of loads applied to the teeth (Ross et al. 2007; Herrel et al. 2008). As teeth are absent in birds, other receptors are probably responsible for feedback. These corpuscles seem to be less abundant in the beak of the chickens described by Lucas & Stettenheim (1972), but can be very abundant at the tips of the beaks of other birds, including sandpipers (Piersma et al. 1998; Nebel et al. 2005) and Ibises (Cunningham et al. 2010), where they are thought to function in prey detection using remote touch. In these species the corpuscles are always found close to the bone or even in small pits in the bone, but at the tip of the beak, rather than at the

lateral aspect of the beak as observed for *Padda oryzivora* and the Black-capped Chickadee (Van Hemert et al. 2012). Although the Herbst corpuscles may play an important role in providing sensory feedback, it remains puzzling that they are absent from areas of the beak known to be under high stress during biting. As such they may have alternate or additional roles in the beaks of seed-cracking birds.

Trabecular organization of the bony beak

Consistent with our predictions, the organization of the trabeculae varies among species in a manner that appears to coincide more or less with variation in their feeding habits. The first group, containing *Geospiza magnirostris*, *G. fortis* and *G. fuliginosa*, typically crush seeds at the base of the beak (Bowman, 1961). The presence of a medial zone with denser concentration of trabeculae at the base of the beak is thus not surprising and may strengthen the beak. Moreover, the diverging trabeculae anterior to the fusion of the os palatinum with the os praemaxillare may play an important role in strengthening the beak as this region is subjected to large tensile forces (bite forces generated by the pterygoid and retractor palatini muscles are transferred to the beak through the os palatinum during seed cracking; Bowman, 1961; Herrel et al. 2010; Soons et al. 2010; Genbrugge et al. 2011). However, it is also noticeable that the smallest ground finch, *G. fuliginosa*, displays a morphology that is almost intermediate between that of Groups I and II. Given that this species predominantly cracks soft seeds, it is not surprising that the trabecular organization of its beak shows some resemblance to that of *G. scandens* or *G. difficilis*, species with probing beak morphologies. Yet, it diverges from the latter species by the presence of a medial zone with dense trabeculae at the base of the beak.

Representatives of the second group, containing *Geospiza scandens*, *G. difficilis*, *Pinaroloxias inornata* and *Certhidea olivacea*, mostly use their beak to probe flowers, foliage, fruits and woody tissues (Bowman, 1961; Grant, 1986). Here, the trabeculae are concentrated laterally at the base of the beak and in the tip, while the middle third of the beak is for the largest part hollow. In *Geospiza scandens* and *G. difficilis* the lateral zones of denser trabeculation are broader and denser than in the other members of this group. This can probably be related to the fact that these finches use their beak for probing as well as crushing of soft seeds (Bowman, 1961; Grant, 1986). The members of the third group, *Camarhynchus psittacula*, *C. parvulus*, *Cactospiza pallida*, are known to use the tip of their beaks to manipulate fruits and insects, rip bark from trees, or handle tools such as needles and small twigs (Bowman, 1961; Grant, 1986). The trabeculae are seemingly randomly organized at the base of the beak but their tip is completely filled with trabeculae, which may be related to their feeding habits involving predominantly the tip of the beak. The sole representative of the fourth group, *Platypiza crassirostris*

tris, shows a morphology combining characteristics from Groups I and III with reinforcements at the base and at the tip of the beak. This corresponds well with the fact that this species uses both the tip and the base of its beak to crush. In general, our results suggest that the regions used most prominently during food manipulation are most variable across species. This is, however, not surprising as the density of trabeculae is often highest in those regions enduring the highest strains (Meyers et al. 2008).

The organization of the trabeculae in the species studied here differs notably from that described for the toucan (Ramphastidae) and the hornbill (Bucerotidae; Seki et al. 2005; Meyers et al. 2008). Inside the bony shell of the beak of the latter species, trabeculae are observed but remain seemingly unorganized and the center of the bony core is hollow. Although a hollow space can also be observed in the species studied here, it is smaller and almost completely lacking in *Geospiza magnirostris* and *Platypiza crassirostris*. In the toucan and hornbill this hollow cavity is thought to function to make the beak lighter (Seki et al. 2005). Given that these birds do not crack seeds, yet transport food by inertial mechanisms, the mechanical constraints on beak function are entirely different and are reflected in a different trabecular organization of the beak. Although trabeculae appear to play an important functional role in strengthening the beak, this remains to be tested explicitly. Given the multitude of selective pressures and constraints operating on beak design (Herrel et al. 2009), the final structure and shape of the beak in Darwin's finches and other birds most likely reflects a compromise phenotype.

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Annelies Genbrugge; Help with methods: Dominique Adriens, Peter Aerts, Joris Dirckx; Critical revision of the manuscript: all authors.

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