Supporting Information

Bickley and Logan 10.1073/pnas.1409913111

SI Results

We measured sternum length, width, and keel height in a range of avian species that have different flight abilities, normalizing for overall body size by dividing sternum measurements by thorax length. We selected hummingbirds, which are highly adapted fliers, pigeons, which are strong fliers that can cover large distances, warblers, and flightless land birds (1). We also selected diving birds including cormorants, which exclusively use their legs for underwater propulsion, and penguins and auks, which both use their wings for underwater propulsion but penguins are flightless, whereas auks can fly (Table S1) (2).

The species selected show a strong positive correlation between keel height and sternum length (Fig. 1; $R^2 = 0.83$). Pigeons and hummingbirds have larger sterna, whereas species we estimate to be poorer fliers and flightless birds have smaller sterna. Most species cluster along a single line of best fit (Fig. 1, lower trend line; $R^2 = 0.89$), which reflects the surface area required for pectoral muscle attachment. We also saw a similar correlation between sternum width and keel height (Fig. S1; $R^2 = 0.58$), although this correlation was weaker than for sternum length. Flightless birds are generally separated from volant birds, occupying the lowest region of the graph and having flatter, shorter sterna. However, three species of flightless birds have relatively larger sterna than other flightless birds (solitaire, kakapo, and dodo; Fig. 1 and Fig. S1, points labeled), which may reflect the reported use of the wings or sternum in display, fighting, or balance (3, 4).

Of the volant diving birds, the auks, cormorants, and penguins lie on the same trend line as the land birds. In the examples of flightless diving birds studied, however, whereas the flightless cormorant lies close to the other flightless birds, the penguins lie closer to the flighted species. A likely reason for this difference is that flightless cormorants use their feet for underwater propulsion, whereas penguins use their wings. Penguins still require powerful pectoral muscles for swimming and, therefore, have a larger sternum and a small keel. The flightless cormorant does not use its wings in the air or underwater, which is reflected by a reduced sternum and absence of a keel.

The hummingbirds lie on a different trend line to the other bird groups studied, having a taller keel but similar sternum length to pigeons (Fig. 1, upper trend line; $R^2 = 0.71$), which may reflect an extreme adaptation to facilitate their unique high-speed wing strokes and ability to hover. Hummingbird flight muscles represent a significantly larger proportion of the total body muscle mass than in other birds and the supracoracoideus muscle is particularly large to generate sufficient force to power the upstroke required for hovering (1). This increased muscle mass requires a larger attachment surface area, generated by a greater sternal keel height. The dimensions of the sternum in avians therefore reflect the mode of locomotion and specifically, the use of the forelimbs. Together these results quantitatively demonstrate the link between the use of the forelimbs in locomotion and the dimensions of the sternum.

1. Warrick D, Hedrick T, Fernández MJ, Tobalske B, Biewener A (2012) Hummingbird flight. *Curr Biol* 22(12):R472–R477.
2. King AS, McLelland J (1975) *Outlines of Avian Anatomy* (Bailliere Tindall, London).
3. Strickland HE, Melville AG (1848) *The Dodo and Its Kindred; or the History, Affinities and Osteology of the Dodo, Solitaire, and Other Extinct Birds of the Islands Mauritius, Rodriguez, and Bourbon* (Benham and Reeve, London).
4. Livezey BC (1992) Morphological corollaries and ecological implications of flightlessness in the kakapo (Psittaciformes, Strigops-Habroptilus). *J Morphol* 213:105.

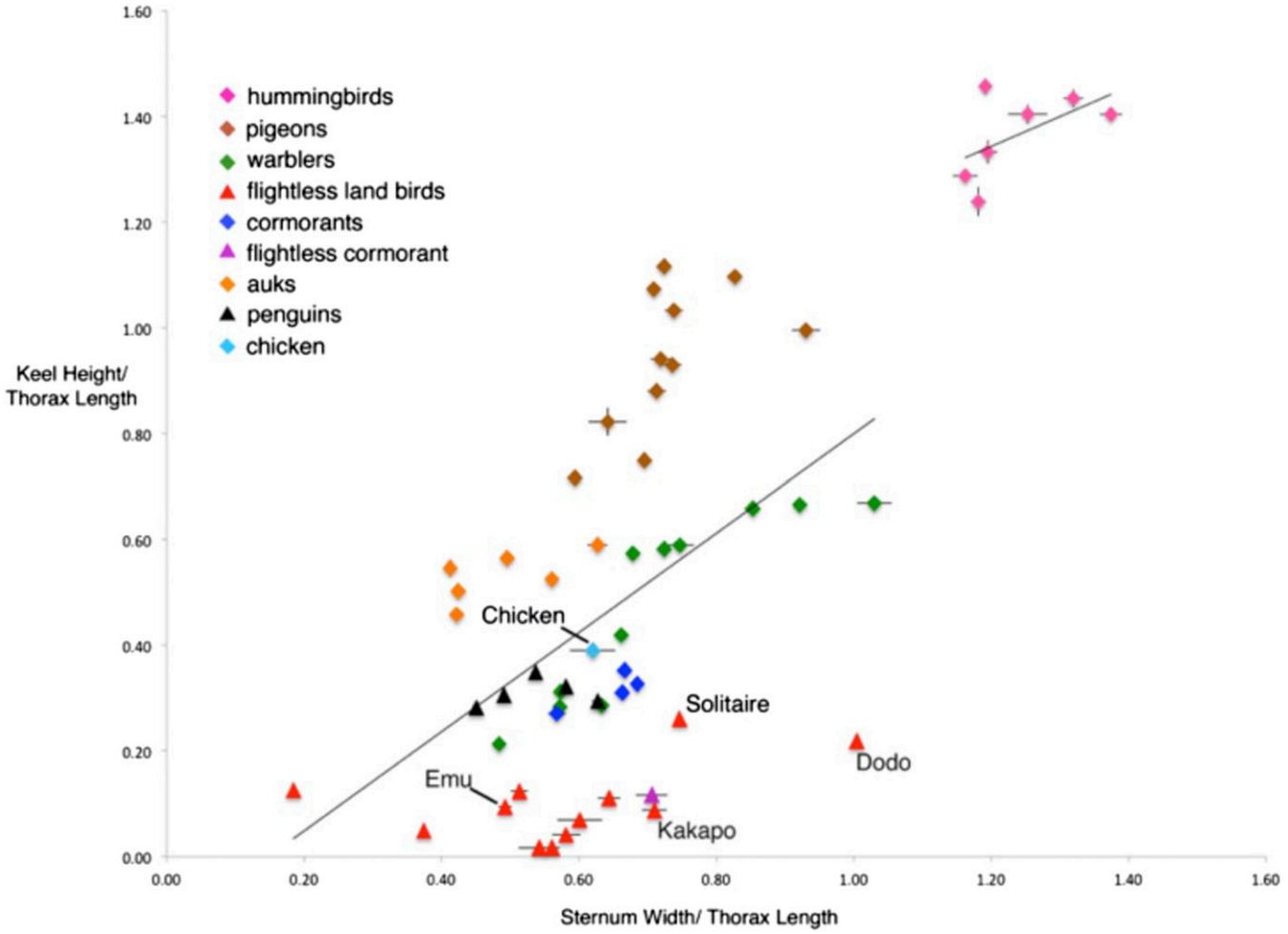
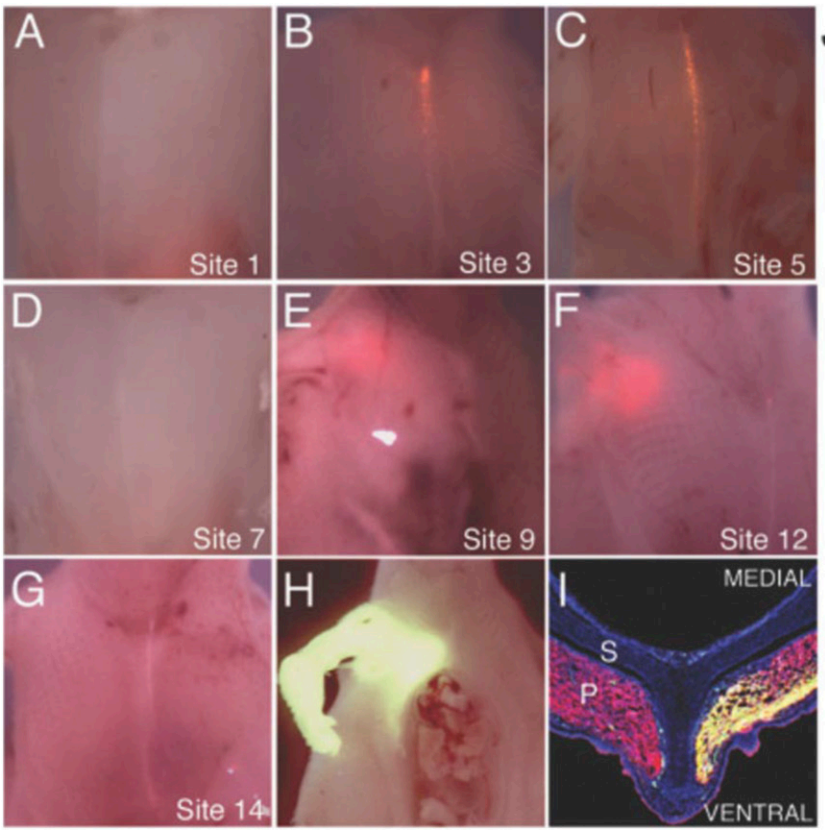


Fig. S1. Sternum width and keel height are correlated with mode of locomotion in avians. Scatterplot showing measurements for sternum width and keel height, normalized for bird size by dividing by thorax length, for a range of bird groups. Each point on the graph represents one species. Where possible, multiple specimens were measured per species. Error bars show SE between multiple specimen measurements. Flying species are represented as diamonds; flightless species as triangles.



J

Injection Site	Embryos with DiI visible	DiI at midline	% of embryos with DiI at midline
1	3	0	0
2	3	2	66
3	5	4	80
4	3	3	100
5	6	6	100
6	4	4	100
7	5	0	0
8	8	0	0
9	7	0	0
10	4	0	0
11	5	0	0
12	7	0	0
13	4	0	0
14	3	0	0

Fig. S2. The sternum precursor cells do not reside within the limb bud proper. (*A*–*G*) Ventral views of harvested and skinned chicken embryos at HH36 following DiI injection at HH20. (*H*) Ventral view of harvested chicken embryo at HH36 following forelimb bud grafting at HH20. GFP-positive cells are visible in the thorax and limb. (*I*) Transverse section through the same embryo followed by immunohistochemical staining for GFP (green) and skeletal muscle (My32, red). P, pectoral muscle; S, sternum. (*J*) Summary table showing percentage of injected embryos with DiI at the ventral midline.

Table S1. List of avian skeletal samples measured, grouped by method of locomotion

Group	Species
Hummingbirds	*Phaethornis superciliosus*
Flying land birds	*Lafresnaya lafresnayi*
	Coeligena torguata
	Heliangelus exortis
	Eriocnemis vestitus
	Metallura therasiae
	Archilochus colubris
Pigeons	*Columba livia*
Flying land birds	*Streptopelia turtur*
	Streptopelia decaocto
	Goura cristata
	Geopelia striata
	Phaps chalcoptera
	Leptotila rufaxilla
	Alectroenas pulcherrima
	Alectroenas madagascariensis
	Phaps elegans
	Gallicolumba luzonica
Warblers	*Sylvia atricapilla*
Flying land birds	*Sylvia comunis*
	Sylvia hortensis
	Acrocephalus schoenobaenus
	Phylloscopus trochilus
	Orthotomus sutoroius
	Locustella naevia
	Sericornis humilis
	Cettia fortipes
	Acrocephalus stentoreus
	Acanthia pusilla
Flightless land birds	*Rhea darwinii*
	Rhea americana
	Dinornis sp.
	Pezophaps solitaria
	Struthio camelus
	Raphus cucullatus
	Casuarios casuarius
	Drominaius novaeholandiae
	Apteryx australis
	Apteryx owenii
	Strgops habroptilus
	Gallirallus australis
Flightless foot propelled diver	*Phalacrorax harrissi*
Cormorants	*Phalacrorax aristotelis*
Flying foot propelled divers	*Phalacrorax carbo*
	Phalacrorax albiventer
	Phalacrorax nigrogularis
Penguins	*Eudyptes crestatus*
Flightless wing propelled divers	*Aptenodytes patagonicus*
	Pygoscellis papua
	Sphiniscus magellanicus
	Mean Sphiniscus demersus
Auks	*Alca torda*
Flying wing propelled divers	*Alle alle*
	Brachyramphus marmoratus
	Uria aalge
	Fraturcula arctica
	Cepphus grylle
Chicken	*Gallus gallus*