ScienceAdvances
Research Article EVOLUTIONARY BIOLOGY

Hoatzin nestling locomotion: Acquisition of quadrupedal limb coordination in birds

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Science Advances 22 May 2019:
Vol. 5, no. 5, eaat0787
DOI: 10.1126/sciadv.aat0787

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One Sentence Summary: The hoatzin nestlings locomotion suggests that bird transitional forms may have use a larger locomotor repertory..

Abstract:

The evolution of flight in birds involves 1) a decoupling of the primitive quadrupedal locomotor coordination, with a new synchronized flapping motion of the wings while conserving alternating leg movements and 2) the reduction of the wing digits and the loss of functional claws. Our observations show that the hoatzin nestlings move with an alternated walking coordination of the four limbs using the mobile claws on their wings to anchor themselves to the substrate. When swimming, hoatzin nestlings use a coordinated motion of the four limbs involving synchronous or alternated movements of the wings, indicating a versatile motor pattern. Finally the proportions of the claws and phalanges in juvenile hoatzin are radically divergent from those in adults, yet strikingly similar to those of *Archaeopteryx*. The locomotor plasticity observed in the Hoatzin suggests that transitional forms that retained claws on the wings could have also use them for locomotion.

Introduction

Birds are flying theropods which power their flight by flapping both wings simultaneously. Indeed, developmental data indicate that the reduction of wing digits and the loss of claws are concomitant during bird evolution *(1)* such that the wings lose their grasping function. Even if some birds such as chukars, ducks, rails, and owls retain claws on the wing *(2)*, they do not use them for locomotion. Hoatzin (*Opisthocomus hoazin*) nestlings, however, retain functional claws on the wing and have been suggested to use them to climb in the vegetation. This is possibly one of the most remarkable, but also least documented traits in this unusual bird. The first description of this behavior was provided by C.G. Young in 1888: "As soon as the young escape from the egg, they creep about with the assistance of these hands, stretching out their wings and digging these claws into hooking on whatever they meet". He further added that a "specimen, by means of these claws walked out of a calabash" (*3*). Another unusual trait in Hoatzin nestlings is to escape by jumping into the water below the nest and to swim back to the vegetation. Although hoatzins are not rare, quantitative data on locomotion in nestlings during either climbing or swimming have never been collected and references to locomotion in these animals all refer back to the original publication on their behavior *(3)*.

Juvenile extant birds may provide key insights in our understanding of the evolutionary and functional transformations that took place towards the evolution of modern birds *(2)*. Before they are capable of active flight, most juveniles flap their wings in the context of wing-assisted incline running (WAIR) to move up steep slopes. During WAIR, the wings generate aerodynamic forces that help the animal ascend obstacles *(5,6)*. As the synchronous wing coordination observed during flying and WAIR is shared by many birds across the majority of clades, it is likely basal for the group (4). The neuronal networks, functionally organized early during development, drive the in-phase movements of the wings during bird locomotion. This determinism is so robust that the experimental substitution of a brachial spinal cord segment by a lumbosacral segment and *vice versa* during the early stages of development in chickens leads to synchronized movements of the limbs connected to the brachial segment of the spinal cord and alternated movements of the wings connected to the lumbosacral segment *(7)*. In that context, the hoatzin is remarkable. Do hoatzin nestlings move using an alternating quadrupedal walk, as suggested by Young's description *(3)*, or do they use the wings and claws in an opportunistic reflex-like way to grasp branches when possible, as when a new-born child grasps a finger *(8)*, or do they use a kind of WAIR behaviour during climbing, as do all other birds? Here we provide the first quantitative data on the locomotion of nestling hoatzins that inform on the use of the claws and the coordination pattern of the limbs. We filmed four nestlings, caught in nests along the Cojedes River in Venzuela, while moving on an inclined substrate and while swimming). Whereas movements were spontaneous in water, nestlings needed to be encouraged to move on the inclined surface by touching their tail or hind feet. The inclined substrate was covered with a towel providing grip for the claw.

Resultss. the limbs moved in alternating fashion with the movement of a leg being followed by the movement of the contralateral wing, then the other leg, and the other wing (Fig. 1). The claws were hooked onto the substrate and the wing flexed, pulling the body upwards. Locomotor cycles were most often irregular as the lack of an immediate

attachment of the claws to the substrate destabilised the nestling bird. When the claw did not hook into the substrate, the motion of the wing continued further laterally untill the claw attached. If it did not, the wing was reversed and a new movement cycle of the same wing was initiated. The quadrupedal locomotion observed was rather irregular with birds stopping typically after two or three cycles. However, the movementes of the four limbs were coordinated . The swing phase duration of the forelimbs was longer than the swing phase duration of the hind limbs (i.e. the wing duty factor was smaller than the foot duty factor). The time lag between the movements was more irregular for the wings than for the legs. However, the tendency is clearly to move the limbs in an alternating way *(9)*(((table 1), with a coordination typical of a quadrupedal walking pattern (FL, HL, PL close to 0.5). This suggests that the use of the wings is not limited to an opportunistic grasping reflex.

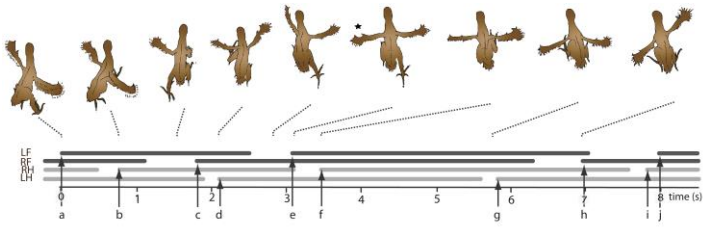


Fig.1: Schematic illustration of a Hoatzin nestling climbing on a 45° inclined surface.. The X-axis represents time. Each line represents the time when a leg is in contact with the substrate. The movements of the four legs are alternating: the left wing moves and grips the subtrate (a). Next, the right foot moves up and touches down (b), followed by the right wing that moves foward (c). The left foot then moves forward and touches down (d), and the left wing moves again (e) followed by the right foot (f). However, the left claw was unable to grip the subtrate at its most forward position (star) resulting in it moving backwards until gripping the substrate (e). The lateral position of the wing perturbates the progression and changes the coordination pattern. The pattern is still alternated but with the left foot (g) moving before the right foot (i) and the right wing (h) before the left wing (j). LF: left fore (wing); RH: rigth hind (foot); RF: right fore (wing); LH: left hind (foot).

The alternating coordination pattern of the wings also does not corresponds to WAIR, where the wings flap in phase to create aerodynamic forces. At hatching, chukars (*Alectoris chukar*) can ascend slopes by crawling on all four limbs (4) but the wings, without claw, cannot anchor to the substrate. No alternated wing coordination has been reported.The hoatzin coordination pattern of the four limbs is typical of a quadrupedal walking gait, a trait likley lost in all other modern birds. This symetrical gait (9) secures at least three points of contact with the substrate and is the most stable of the quadrupedal coordination patterns.

When placed in the pool, the nestlings swam vigourously and with great ease, either under water or with the head kept outside of the water. Irrespective of the coordination, the swimming cycles were rather regular, even if a bit more variable for the wings compared to the legs. The wing power phases were shorter than the recovery phases whereas

they were longer for the legs. The coordination between the leg and the wing (PL) was variable (high standard deviations). The movements of the legs were alternated (HL close to 0.5) while the wings typically moved in phase (FL close to 0, table 1) (Fig. 2). Out of the 50 locomotor cycles oberved, only four of them showed an out of phase coordination pattern (l).The coordination during most swimming cycles was thus generally similar to that observed during WAIR in other birds, but in a different, mechanical context.

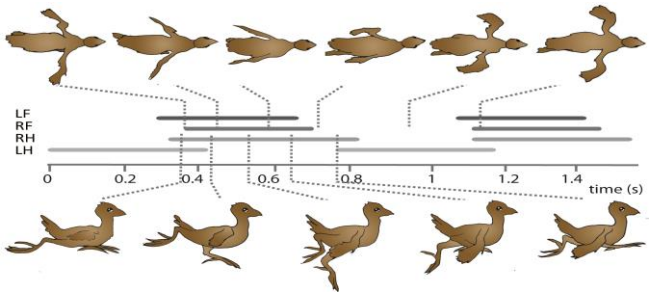


Fig. 2: Schematic illustration of a hoatzin nestling swimming. The X-axis represents time. Each line represents the propulsive phase, when the limb is moving backward. The dorsal view shows a synchronised motions of the wings; the lateral view shows the alternated motion of the limbs. LW: left wing; RW: right wing; LF: left foot; RF: rigth foot.

In a more complex environment with branches, hoatzin nestlings used a quadrupedal walking coordination but due to the irregularity of the substrate, the coordination was far less regular than on our experimental substrate. The head was also used as a hook: it was flexed so that the base of the beak was positioned on the branch, the neck appearing to pull the body upwards and helping the wings. The claws on the fingers were actively moved independent of the movements of the rest of hand skeleton. Contrast-enhanced micro CT images of a late stage embryo show that the hoatzin has multiple muscles and tendons attaching onto the finger bones as observed in most other birds *(10-13)*. However, an additional tendon of one of the digital flexor muscles attaches onto the distal phalanx of the alula (Fig. 3). This likely allows the active gripping of the branches by the claws. A comparison of the proportions of the phalanges of the hoatzin nestlings with those of *Archaeopteryx (14)* shows a remarkable similarity in proportions between the two (Fig. 4),. The proportions in adult hoatzin are, however, quite different from those observed in nestlings.

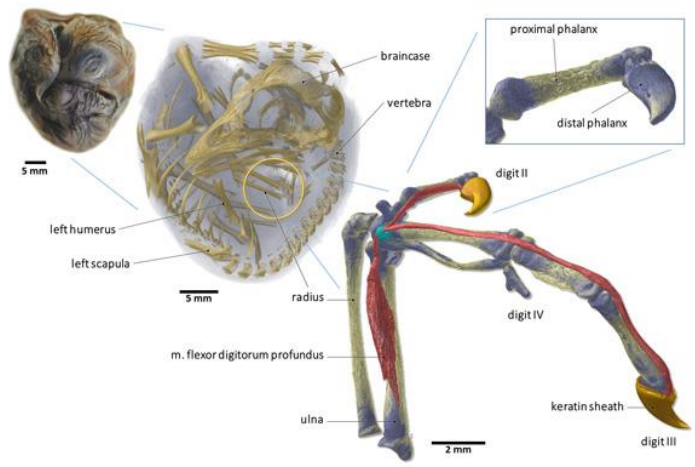


Fig.3: Musculoskeletal anatomy of a hoatzin shortly before hatching . Fetus as positioned in the egg shown on the left). Centre image: reconstructed mineralized parts of the skeleton of the bird, showing the position of the wing skeleton (yellow circle). Right: detailed reconstruction of the contrast enhanced µCT data of the wing (ventral view), with the position of the additional tendon of the *flexor digitorum profundus* attaching to the alula digit illustrated. Insert: detail of the alula digit with the keratin sheet removed, showing the claw-like distal phalanx. blue, cartilage; yellow, bone; red, muscle; cyan, connective tissue sling of the muscle tendon; orange, keratin.

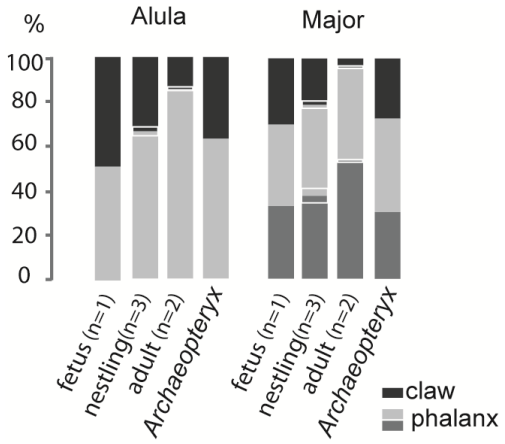


Fig. 4: Proportions of the digit phalanx in the *Archaeopteryx* compared to three Hoatzin developmental stages. Values in percent of the digit length, variability shown with white line.

Discussion

Quadrupedal locomotion requires a coupling of the forelimbs, of the hind limbs, and a coupling between the limb-pairs at the level of the spinal neuronal network *(9, 15)*. In vertebrates, locomotion is initiated at the level of the brainstem and generated by a central spinal network *(16)*. In mammals, which are able to use in-phase and out-of-phase movements for each limb-pair, two sets of commissural interneurons are involved in the right-left coordination. An inhibitory pool of neurons is activated for alternating, out of phase, coordination, and an excitatory pool is activated for synchronous, in-phase coordination *(17)*. Their interplay depends on the behavioral context and the associated locomotor speed. In birds, the neural network is organized early during development *(7)*, and triggers in-phase movement of the wings. The in-phase flapping of the wings could thus have arisen from either the loss of the inhibitor commissural neuron pool, or its silencing, The Hoatzin nestlings exhibit both in-phase movements during swimming and out-of-phase movements during climbing. This suggests that they have both excitatory and inhibitory connections between the interneuronal networks of the limbs. The plasticity exhibited in the coupling between the excitatory and inhibitory connections in the Hoatzin nestling could then arise either from descending drive or from the effects of proprioceptive feedback, or both. The quadrupedal coordination goes hand-in-hand with the presence of functional claws on the wing *(1)* since without claws the wings cannot anchor the body to the substrate and would thus be unable to generate the locomotor forces. During slow movements the locomotor mechanics require at least three anchoring points for stability, preventing the coordination of wings into an in-phase motion. Proprioceptive feedback may participate in the reactivation of a silent inhibitory motoneuron pool during quadrupedal locomotion.

Birds originate from theropods, bipedal animals which did not use the forelimbs for walking. Although the exact position of the hoatzin in the bird tree of life remains controversial *(18-22)*, its divergence seems to have occurred after the origin of Paleognathes, Galloanseres, and other neoavian radiations *(22)*. None of the species of these clades are known to use the wings for climbing. Furthermore, the forelimb in-phase coordination is determined early in the development in the chicken (*Gallus gallus*) *(7)*, a Galloanseres species from a clade more basal than the Opisthocomiformes *(22)*. The quadrupedal walking coordination of the hoatzin nestling thus represents the re-appearance a trait lost during bipedal saurischian dinosaur evolution *(23)*, without the loss of a trait that has arisen later in the evolution of birds (wing flapping during flight, retained in adult hoatzin). The quadrupedal coordination may be the expression of the conservative nature of the central nervous system, with a basic interneural network re-activation in response to proprioceptive feedback, driven by the contact of the claws to the substrate. It is possible that the interneuronal networks show greater plasticity and diversity among birds than has been previously recognized,

due to a sparse sampling of 'model animal' species in neurophysiological studies. As *Archeopteryx* shows large claws on the wing similar in proportion to those observed in the Hoatzin nestlings, the latter might be used as a functional analogue to infer the locomotor repertoire in transitional forms like *Archaeopteryx*. Our results thus suggest the existence of a larger locomotor repertoire in transitional forms likely including both WAIR wing flapping and quadrupedal limb coordination during climbing allowed by the presences of claws on wings (24).

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Materials and methods

Animals and filming

Animals were caught on the in October, 2014 along the Cojedes river near the town of El Baul under permit number 950 issued by the Venezuelan government. Animals were transported back to the field laboratory and filmed with three HDR-CX740VE Sony cameras at 50Hz. Animals were induced to climb up an inclined surface covered with a cloth to provide grip then climb on branches. Subsequently animals were induced to swim in an aquarium (100 x 50 x 50 cm) with a water depth of 15 cm. All the procedures were approved by the ethics committees of the MNHN (Comité Cuvier) and of IVIC (COBIANIM).

µCT scanning

A late stage hoatzin embryo (egg length 4.1 mm), four juveniles and two adults were µCT-scanned at the "Centre for X-ray Tomography" at Ghent University (UGCT). A first *in toto* scan of each specimen was performed to get a complete overview of the mineralized skeletal anatomy using the in-house developed HECTOR scanner (*25)*. A total of 2400 X-ray projections over 360° were taken at 120 kV tube voltage and 20W target power with the PerkinElmer detector (pixel pitch 0.2 mm, exposure time 1000 ms per image) yielding an isotropic voxel pitch of 20 µm. Subsequently, the left wing was cut off of the late stage embryo and transferred to ethanol 50% and PBS (1 hour), after which it was treated with 2.5% phosphomolybdic acid for one week, to visualize soft tissues with µCT. The wing was then gradually transferred back to ethanol 70% and scanned at HECTOR under similar settings (but at 100kV and 10W), at an isotropic voxel pitch of 10 µm. Virtual cross sections were reconstructed using the in-house developed software Octopus (version 8.8.2.1; *(26)*). Bone and soft tissues were segmented and visualized using Amira (version 6.0, FEI). Proportions of the phalanges and claws in *Archaeopteryx* were measured based on the illustrations in Griffiths *(14)*.

Gait analysis

Climbing: On the videos, we noted the time when the limbs gripped the cloth and stopped moving as well as the time when the claws were released from the cloth. Even if the delays between the movements may be long and the coordination perturbed by additional grips, the coordination remained similar across the more than 20 locomotor cycles analysed: the movement of a wing was followed by the movement of the opposite foot, then the other wing moved followed by the other foot. Finally, the first wing moved again (Fig. 1). The movements were, however, very

slow and irregular. For our quantitative analysis we kept only the cycles with stance phases lasting less than ten seconds and swing phases less than two seconds. As the birds often stopped, we did not always have two successive complete cycles so that we calculate the gait parameters for each limb even if it was not possible to quantify all the parameters for all of them in a given cycle. The swing phase was defined as the time when the limb is off the substrate; the stance phase was defined as the time during which the claw gripped the cloth. Cycle duration was quantified as the sum of the swing phase duration plus the stance phase duration. The duty factor was defined as the participation of the stance to the total cycle duration (i.e. the stance duration divided by the cycle duration). We also calculated coordination parameters (*27)*: the fore lag (FL) was defined as the time lag between the beginning of the two wing stance phases. The hind lag (HL) was defined as the time lag between the beginning of the two foot stance phases. Finally the pair lag (PL) was defined as the time lag between the stance phase of a wing and the stance phase of the ipsilateral foot.

Swimming: fifty swimming cycles were observed. In four of them the wings moved in phase. In all the other case, the wings and the feet moved out of phase. We observed different coupling (Fig. 2) between the forelimbs and the hind limbs. Due to the constraints of the field experiments we were not able to quantify all the cycles observed. We selected the sequences when the birds moved parallel to the camera in lateral view allowing us to see the motion of both the hind limbs. The motion of the wings was visible but not accurate enough to be measured on the lateral view. The two wings were clearly visible on the dorsal views, but the hind limbs were often hidden by the wings or by reflections on the water. We selected sequences where it was possible to synchronize the motion of the wings and the legs for our quantitative analysis. We considered the power phase of a limb the phase when it moved backwards and the recovery phase when it moved forwards (hind limbs) or laterally (wings).

Author contributions

AA conceived the project; MAGA organized the field work; AA, AH, TD, ACF, MAGA participated in the field work and the capturing and filming the animals; AA, AH, TD, DA, FP analyzed the data; LVH was responsible for the µCT scanning of the specimens; DA and FP segmented the µCT data; AA, AH wrote the paper, all authors revised the paper. All authors declare that they have no competing interests

Acknowledgements

The authors would like to thank Jose González-Fernández from Hato Mataclara and the Hato Pinero staff for their support in the field, J.R. Cazalets and Ph. Janvier for their remarks on the manuscript, D. Geffard-Kuri for help with the illustrations. We thank the Ministerio del poder popular para la ecosocialismo, habitat y vivienda for the capture and exportation permits. Funding by ATM MNHN, PEPS ExoMod CNRS (AA) and IVIC grant No 67 (MAGA). All data needed to evaluate the conclusions in the papers are present in the paper.Additional data related to this paper may be requested from the authors.

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Table 1: gait characteristics and limb coupling during climbing and swimming.

Climbing											
	cycle duration (s)				duty factor				FL	HL	PL
	RF	LF	RH	LH	RF	LF	RH	LH			
Mean	4.20	3.10	5.58	6.31	0.86	0.83	0.94	0.96	0.36	0.48	0.56
Std.	1.61	2.27	2.25	2.97	0.10	0.05	0.04	0.01	0.33	0.10	0.22
N	11	10	12	11	10	7	10	11	8	9	9
Swimming In-phase coordination											
Mean	0.77	0.82	0.75	0.74	0.43	0.41	0.54	0.53	0.05	0.42	0.32
Std.	0.18	0.18	0.06	0.07	0.08	0.11	0.08	0.04	0.07	0.09	0.18
N	12	12	15	13	12	12	15	13	9	9	10
Out of phase coordination											
Mean	0.72	0.72	0.71	0.7	0.34	0.31	0.62	0.53	0.52	0.52	0.18
Std.	0.16	0.06	0.07	0.08	0.05	0.07	0.07	0.06	0.13	0.12	0.16
N	4	4	4	4	4	4	4	4	4	4	4

FL, fore lag; HL, hind lag; N, number of cycles analyzed; PL, pair lag; Std, standard deviation.

Movies S1-S#

Videos of the experimental conditions, climbing and swimming in hoatzin nestlings.