The Origin of a Curved Beak

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New York Medical College

Embryo Physics course
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Wilhelm His
(1831-1904)

Embryology and morphology cannot proceed independently of all reference to the general laws of matter, to the laws of physics and of mechanics. This proposition would, perhaps, seem indisputable to every natural philosopher; but, in morphological schools, there are very few who are disposed to adopt it with all its consequences (His 1888)

Cited by R. Gordon in the preface of the book
Mechanical Engineering Of The Cytoskeleton In Developmental Biology

Bird beak

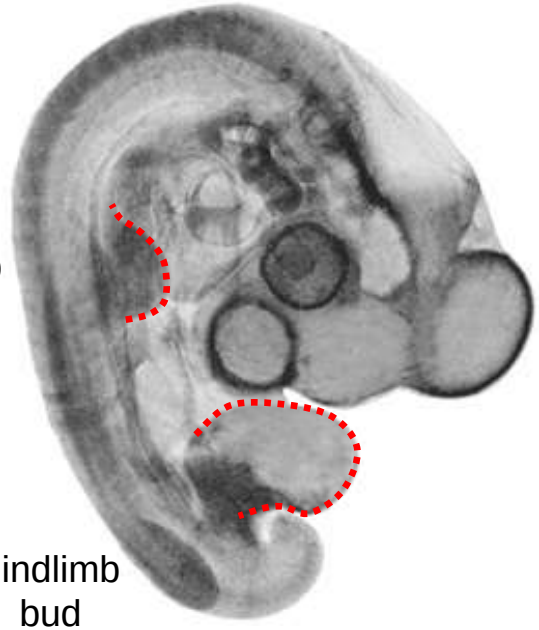
Wide diversity of forms

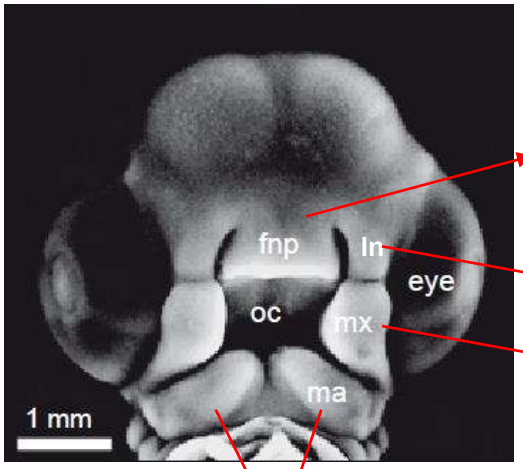
Paradigmatic Darwinian system

A multi-primordium organ

Limbs

Beak





Frontonasal mass (1)

Lateral nasal (2)

Maxillary (2)

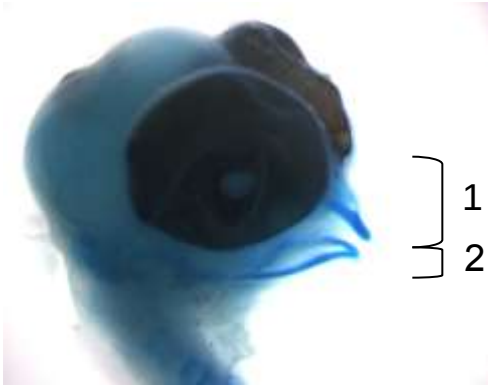
Upper beak (5)

Mandibular (2)

Lower beak (2)

Beak skeleton

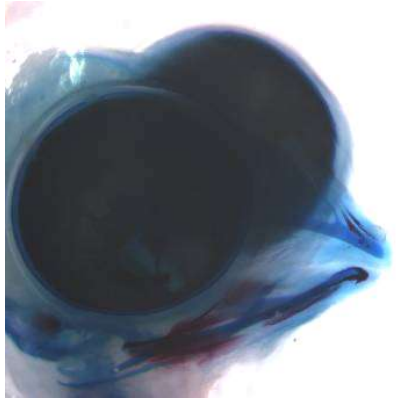
E9



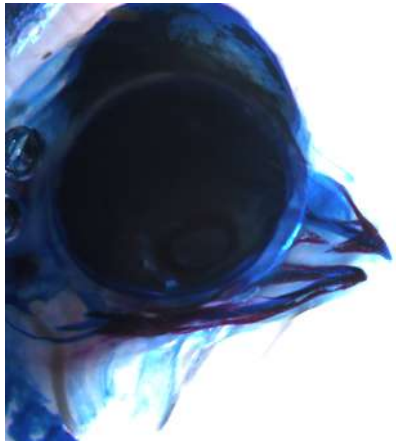
1 rod: Nasal cartilage

2 rods: Meckel's cartilage

E10



E11

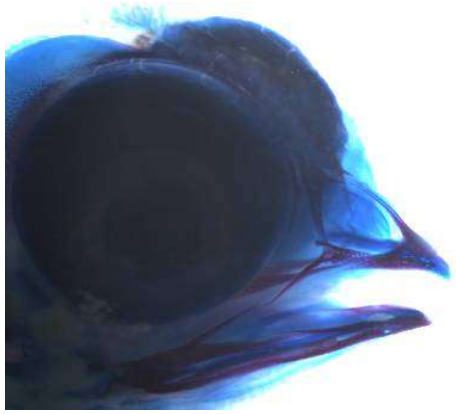


Alizarin-Alcian blue staining

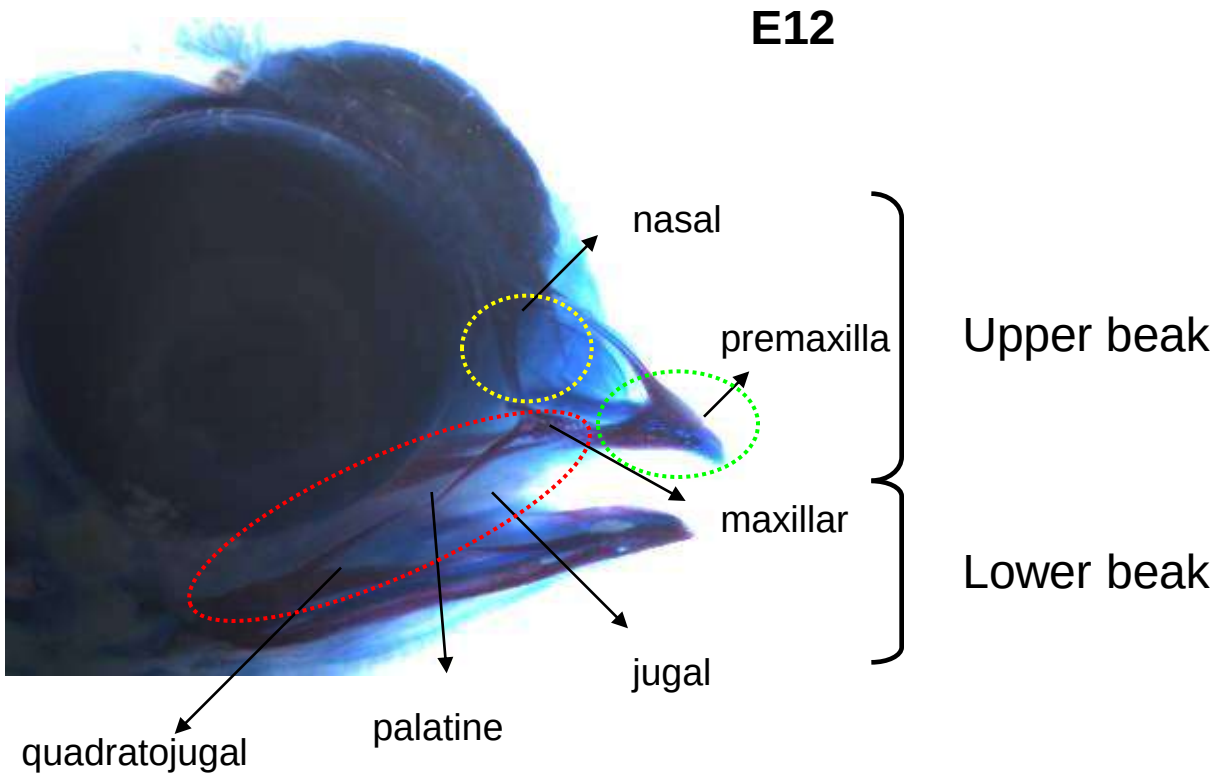
Cartilage

Membrane bones

E12



Different bones arise from different buds



Gene-centric model - 1

Two developmental modules establish 3D beak-shape variation in Darwin's finches

Ricardo Mallarino[a], Peter R. Grant[b], B. Rosemary Grant[b], Anthony Herrel[c], Winston P. Kuo[a,1], and Arhat Abzhanov[a,2]

[a]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138; [b]Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ 08544; and [c]Département d'Ecologie et de Gestion de la Biodiversité, Muséum National d'Histoire Naturelle, 75231, Paris Cedex 5, France

Basic idea

1. Different beaks are the result of different skeletons

2. Different skeletons are the result of different gene expression patterns

Gene-centric model - 2

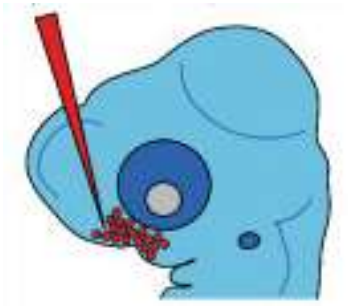
Candidate genes: different expression patterns in different finch species

BMP4, Calmodulin, TGFβRII, β-catenin and Dkk3

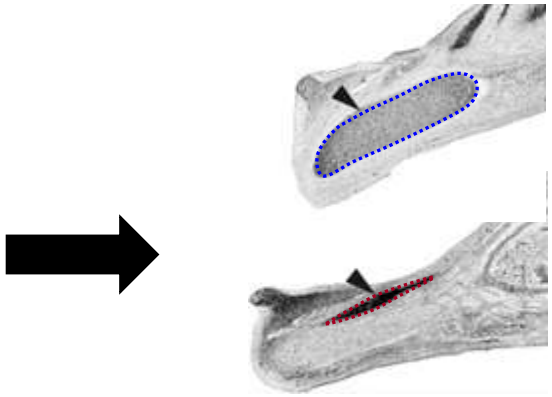
Experiments in chicken embryos

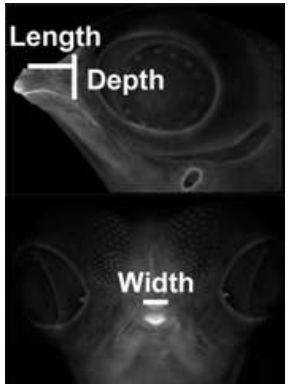
Gain-of-function experiments **Cartilage/bone staining** **Length measurements**

Inject retrovirus



Schneider 2006



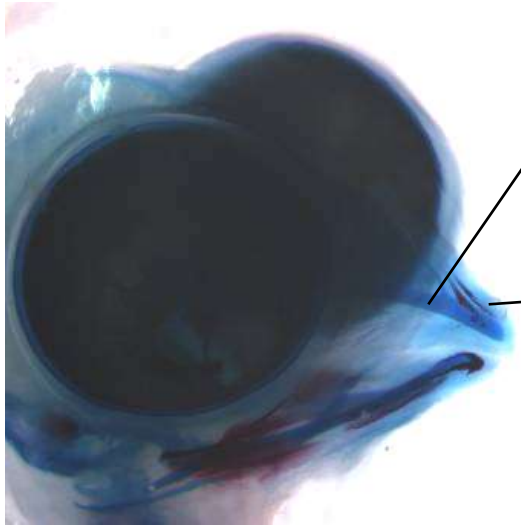


Mallarino et al 2011

E4 **E11**

Gene-centric model - 3

Two independent developmental modules



Cartilage

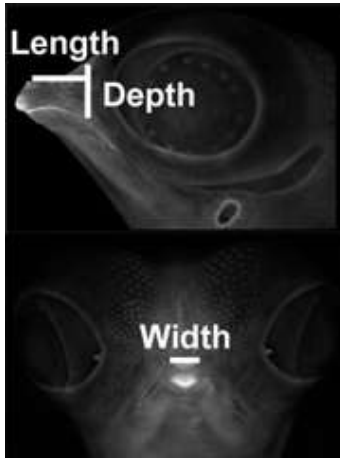
BMP4
Calmodulin

Bone

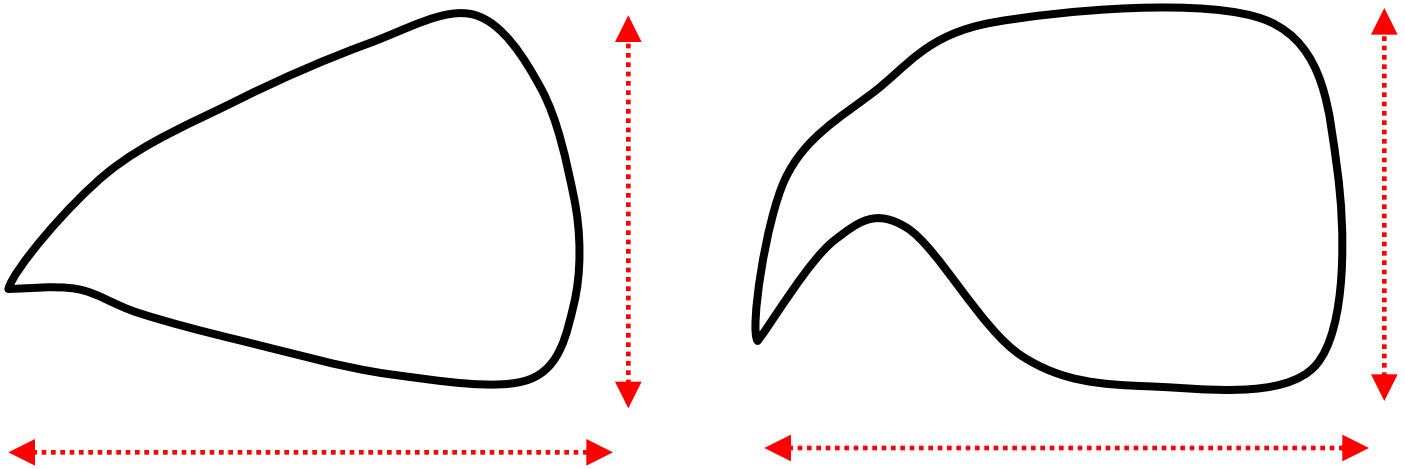
TGFβRII, β-catenin and Dkk3 network

Gene-centric model - 4

Expression	Beak dimension
BMP4	Depth, width
Calmodulin	Length
TGFβRII, β-catenin and Dkk3	Depth, length

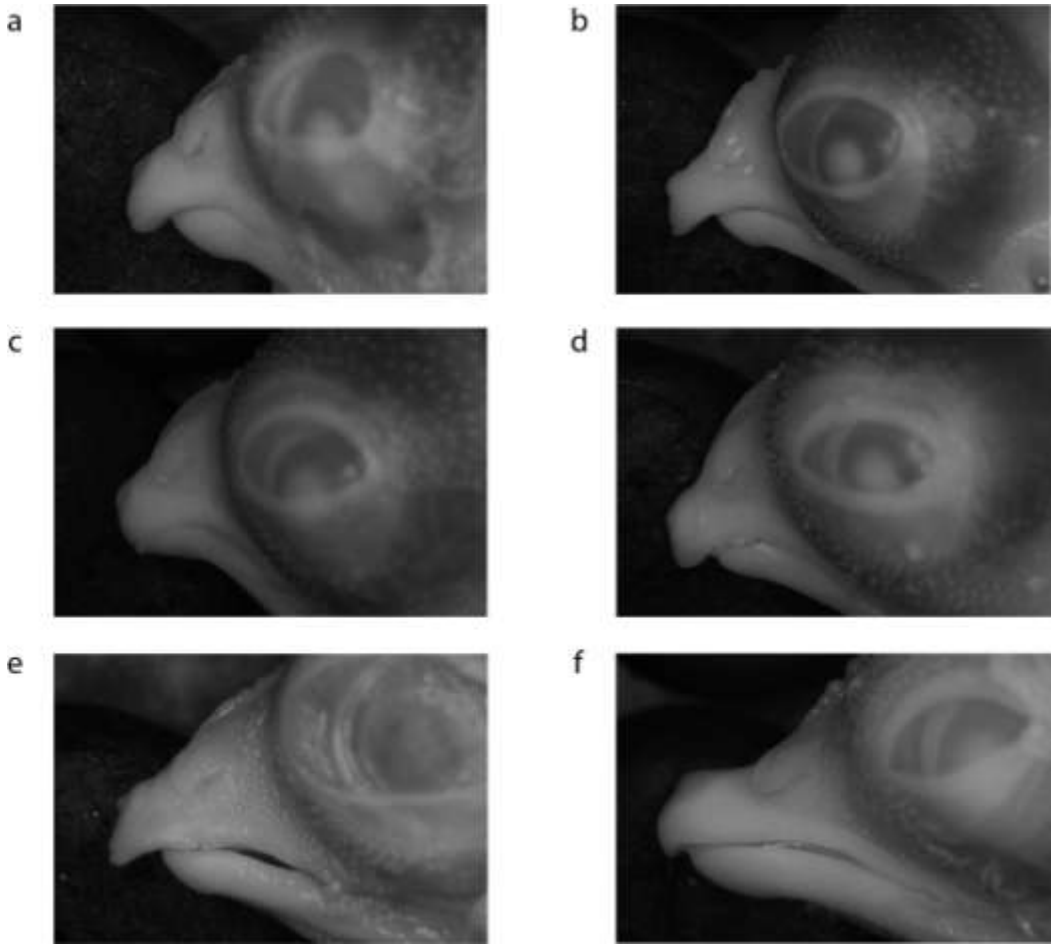


Conventional morphometrics



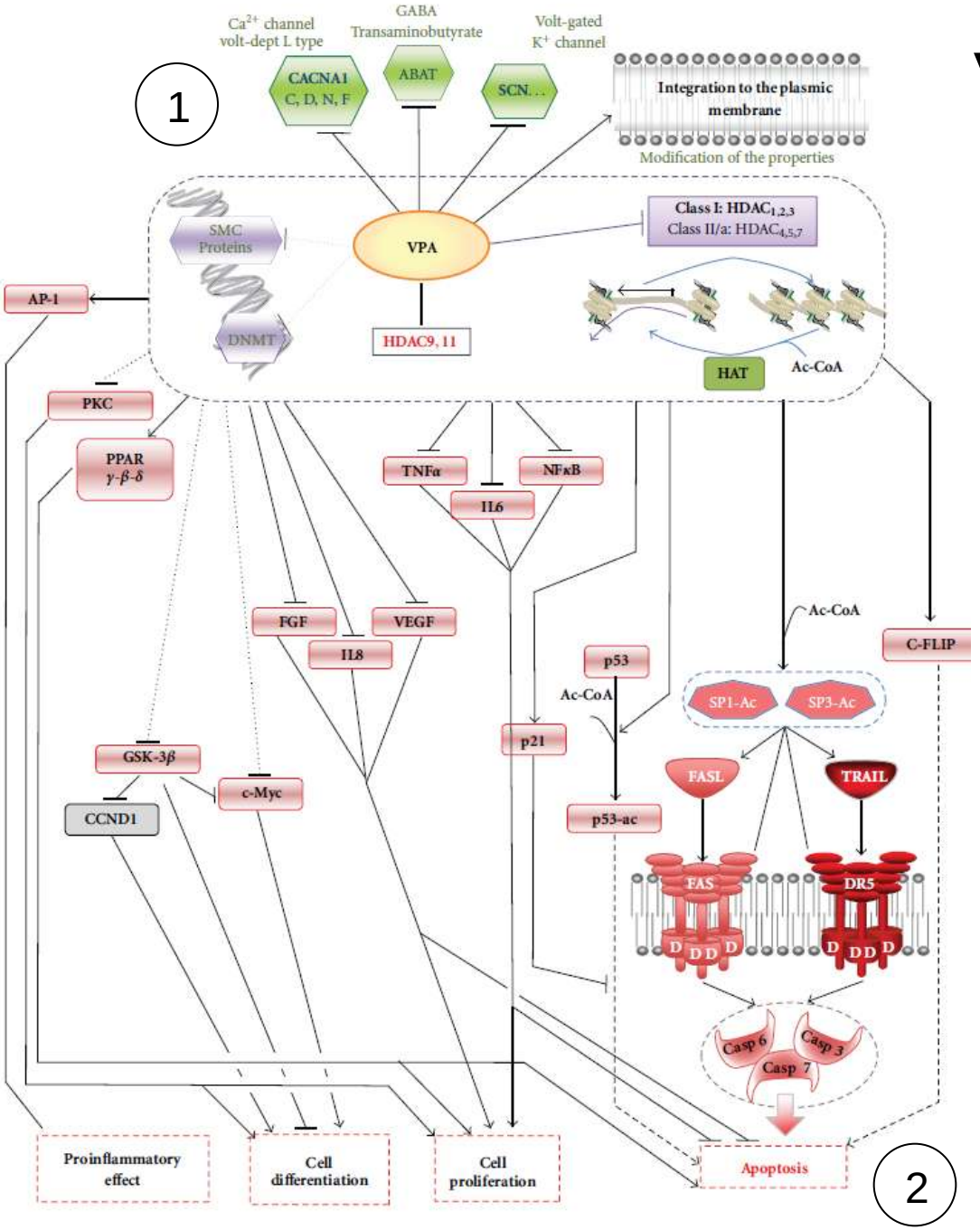
These hypothetical beaks have the same length and depth, but present substantial shape differences. A conventional morphometric analysis do not capture this shape variation.

A curved beak is a form within the plastic repertoire of the chicken embryo



Induced curved beaks at E13 by the injection of different teratogens (at E4): (a) 3-acetylpyrimidine, (b) boric acid, (c) DAPT, (d) sulphanilamide, (e) valproic acid; (f) an untreated specimen is showed for allowing comparisons. Pictures are at the same scale.

Valproic acid (VPA)



1. Antiepileptic drug

2. Teratogen. It is an oxidant agent that produces a decrease in embryonic growth and an increase in neural tube defects by increasing apoptotic levels (Tung and Winn 2011). It also alters cell proliferation and cell differentiation.

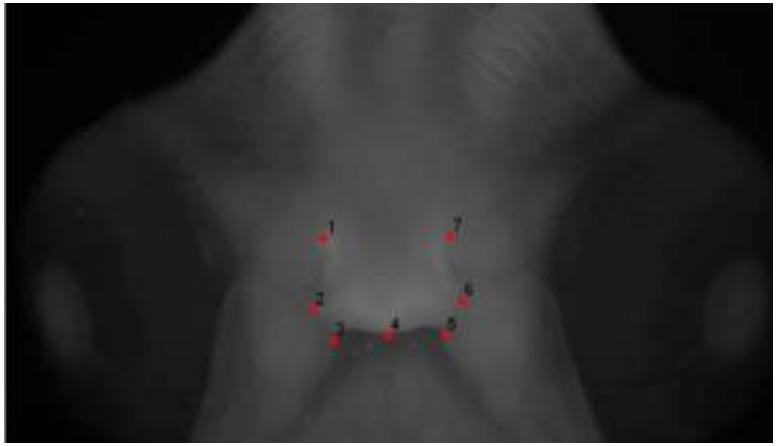
Chateauvieux et al. 2010

Valproic acid experiments

Systemic administration

E4

Geometric morphometric analysis

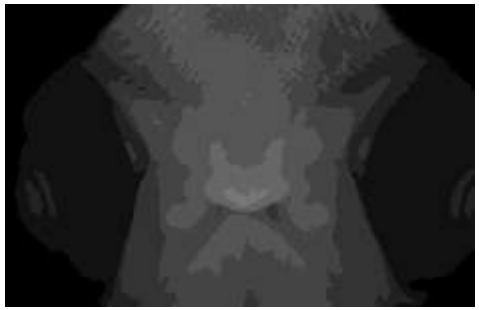


From E5 to E12

Geometric morphometric analysis - 1

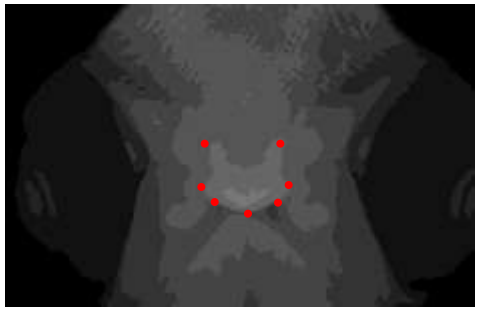
1

digital images



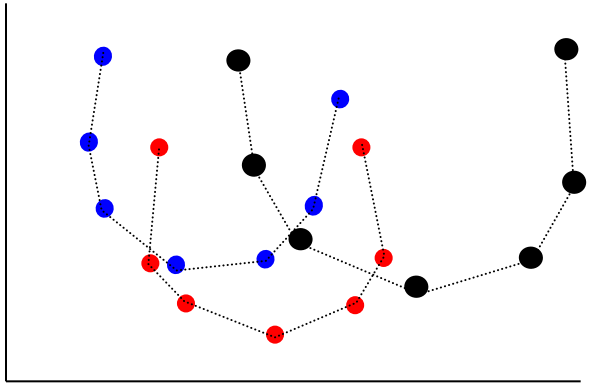
2

xy coordinates of landmarks



3

raw data



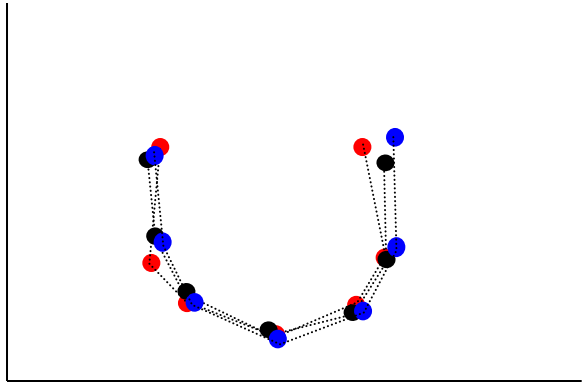
shape differences
+
arbitrary variation

scaling
translation
rotation

Procrustes analysis

4

new *xy* dataset



shape differences

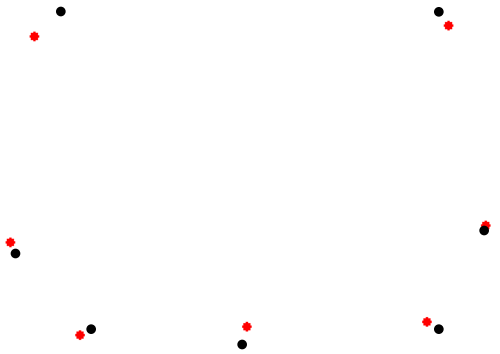
Geometric morphometric analysis - 2

all specimens

consensus

5

pair comparisons



Fit data to *thin-plane spline* function

The parameters of this function are the **shape variables** for each specimen

Geometric morphometric analysis - 3

6 W → Conventional statistical tests

Matrix of shape variables

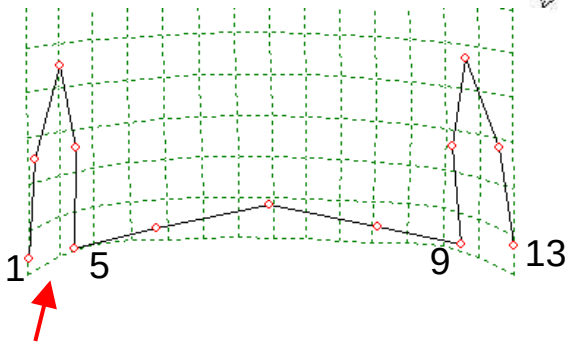
Principal Component Analysis (PCA)

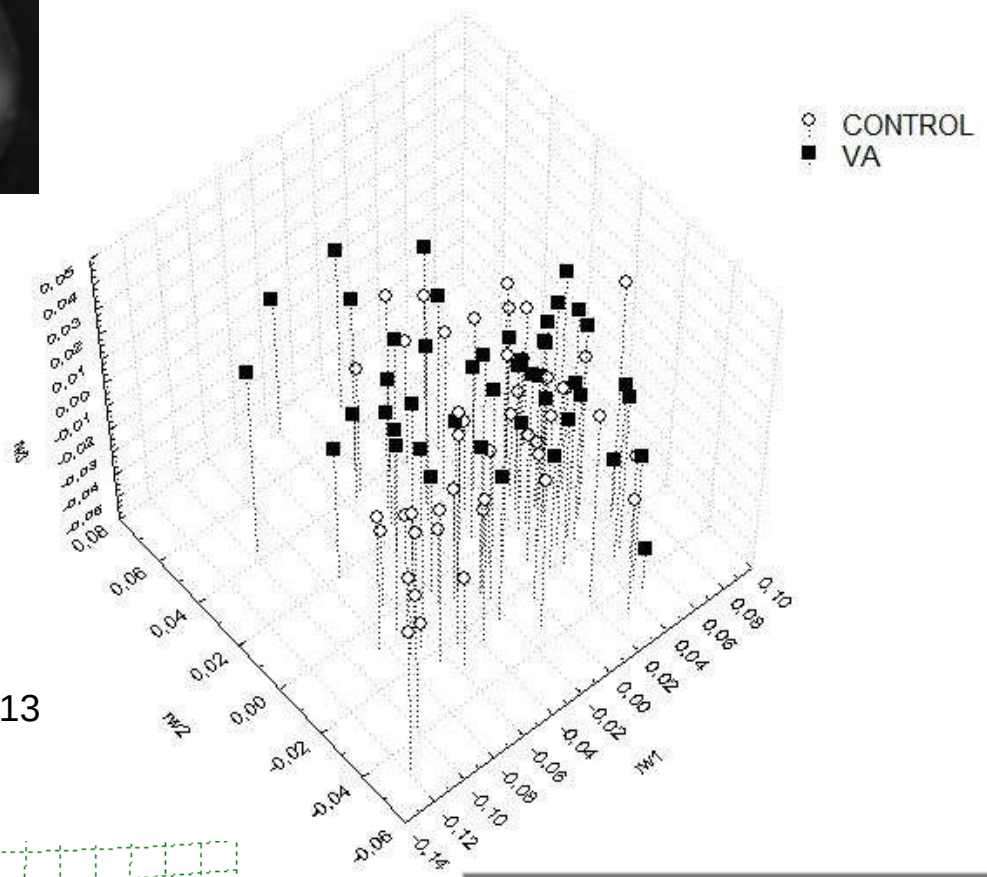
Main shape patterns

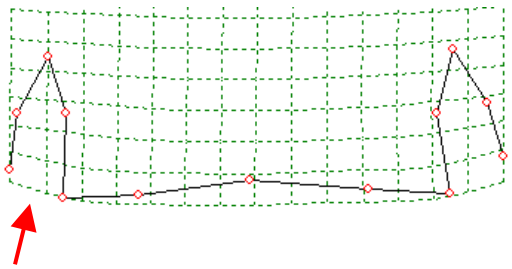
MANOVA

Shape differences between control and treated embryos

Results -1

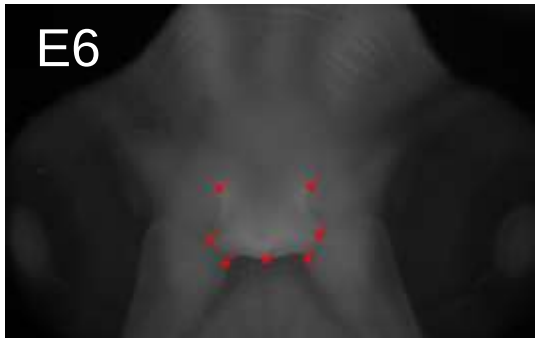




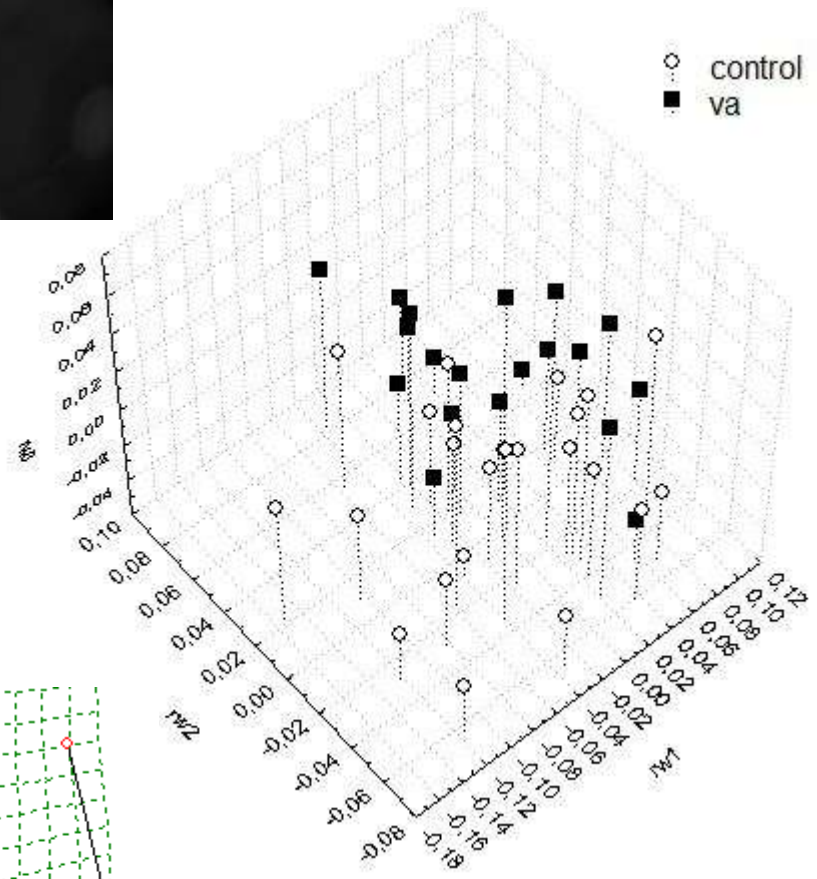


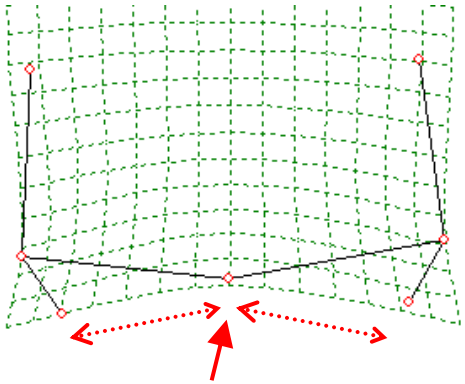
MANOVA table

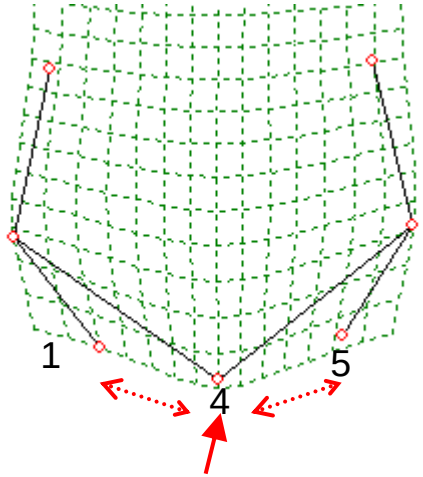
Effect	Multivariate Tests of Significance (wm) Sigma-restricted parameterization Effective hypothesis decomposition					
	Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0,999918	0,000253	22	68	1,000000
treatment	Wilks	0,596460	2,091176	22	68	0,010955



Results - 2



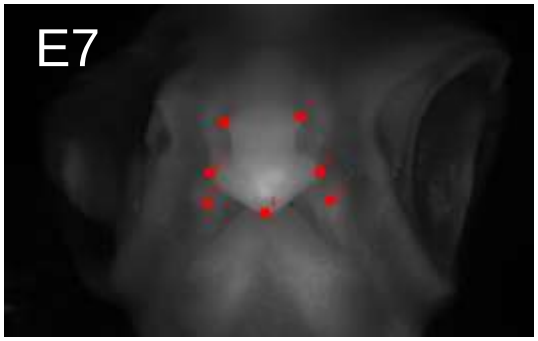


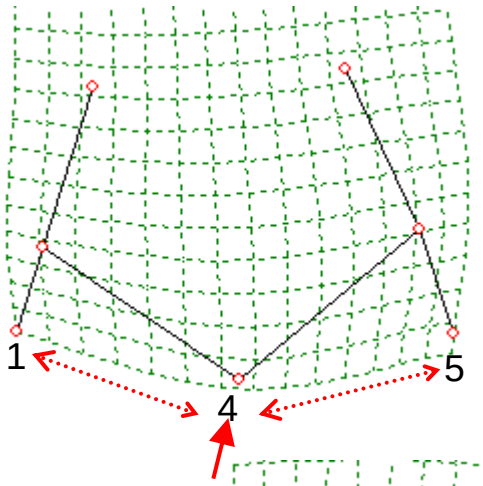


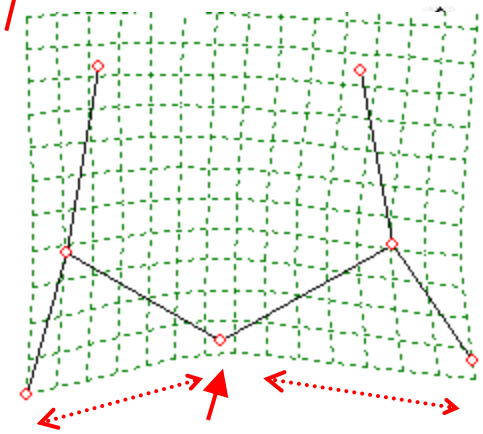
MANOVA table

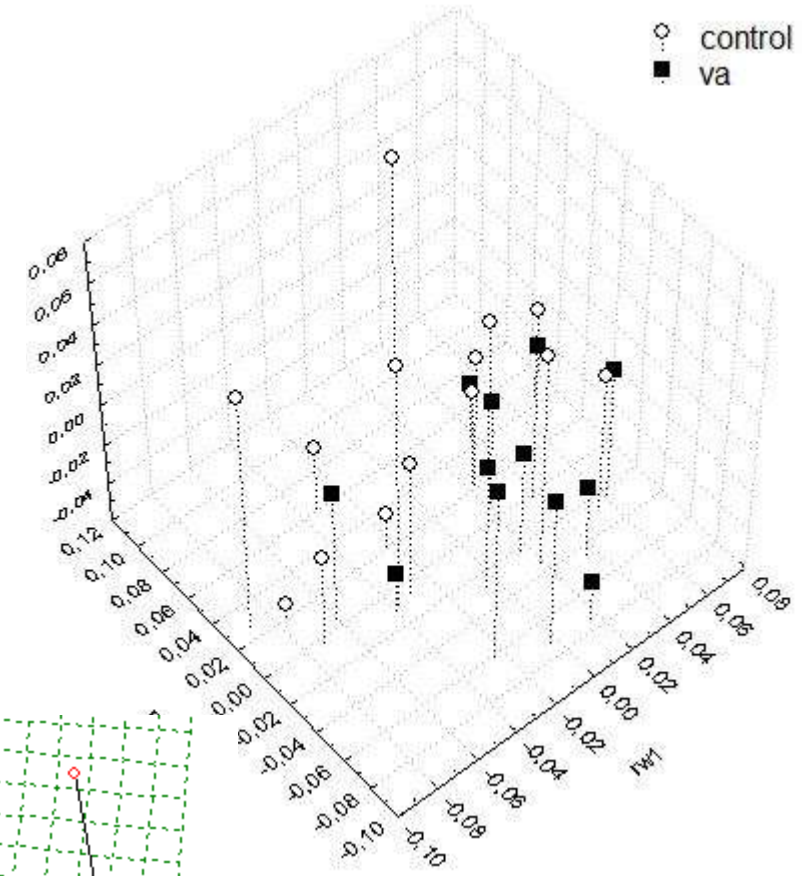
Effect	Multivariate Tests of Significance (wm) Sigma-restricted parameterization Effective hypothesis decomposition					
	Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0,986207	0,044756	10	32	0,999993
treatment	Wilks	0,491663	3,308523	10	32	0,004784

Results - 3







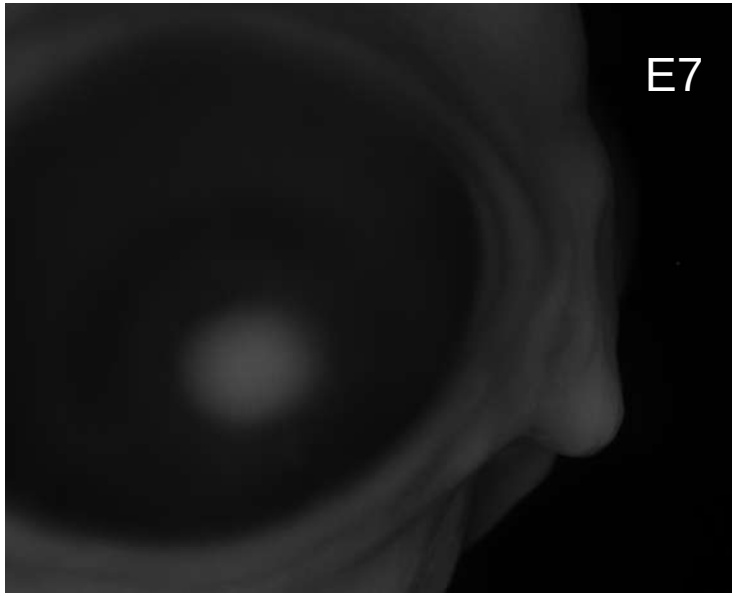


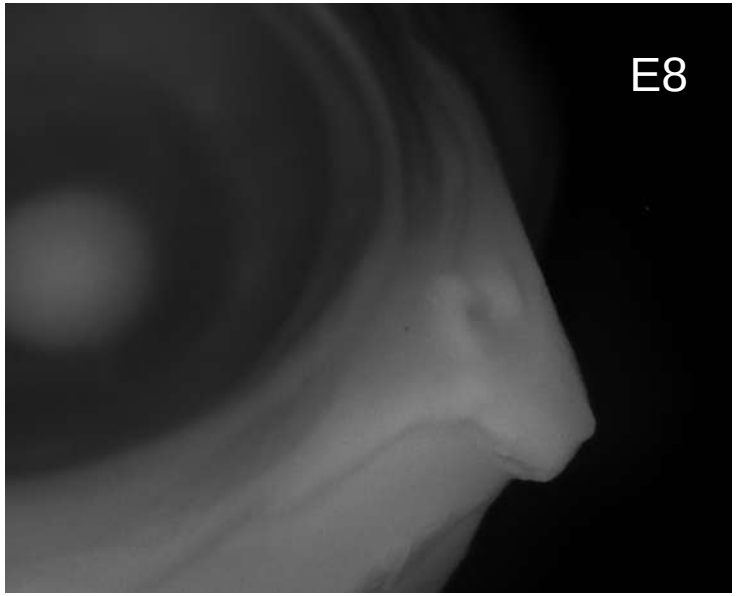
MANOVA table

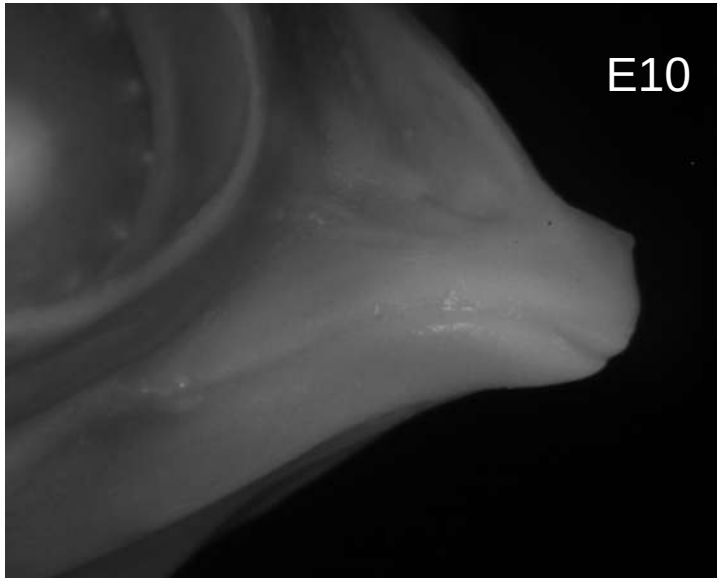
Effect	Multivariate Tests of Significance (wm_excel) Sigma-restricted parameterization Effective hypothesis decomposition Test	Value	F	Effect df	Error df	p
Intercept	Wilks	0,988141	0,018001	10	15	1,000000
ltreatment	Wilks	0,329935	3,046348	10	15	0,025453

Interpretation

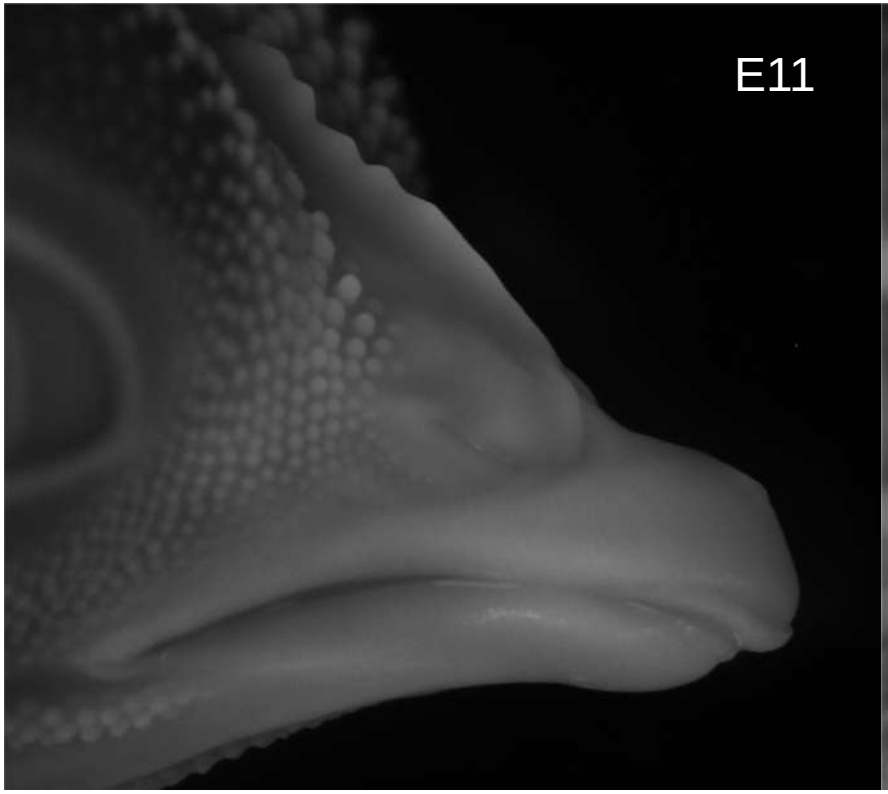
VPA-treated embryos have less expanded buds, their tips are more separated from each other, and consequently, the oral gap is larger

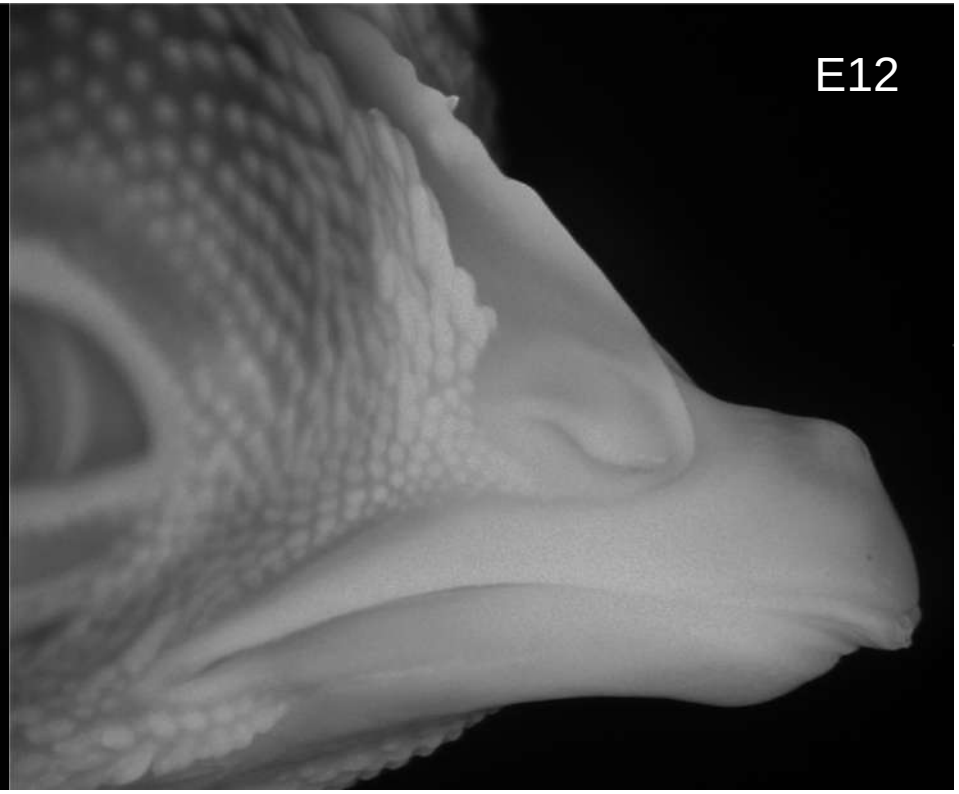






CONTROL





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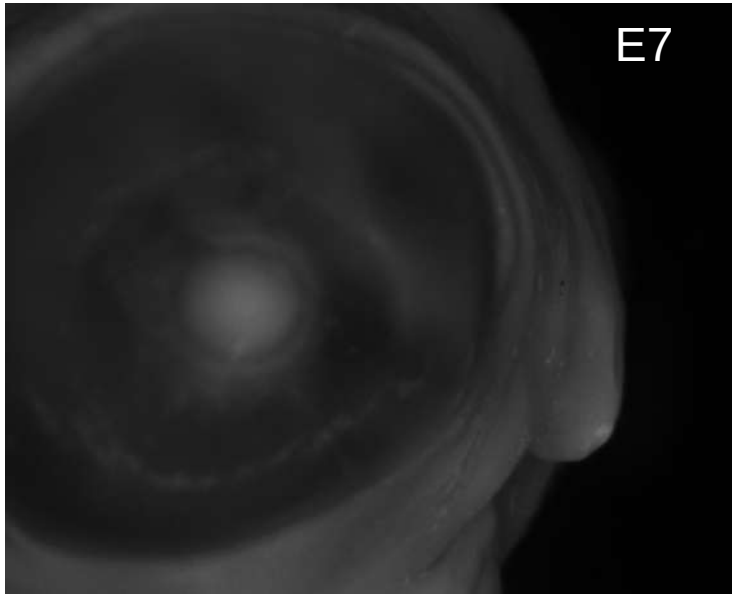
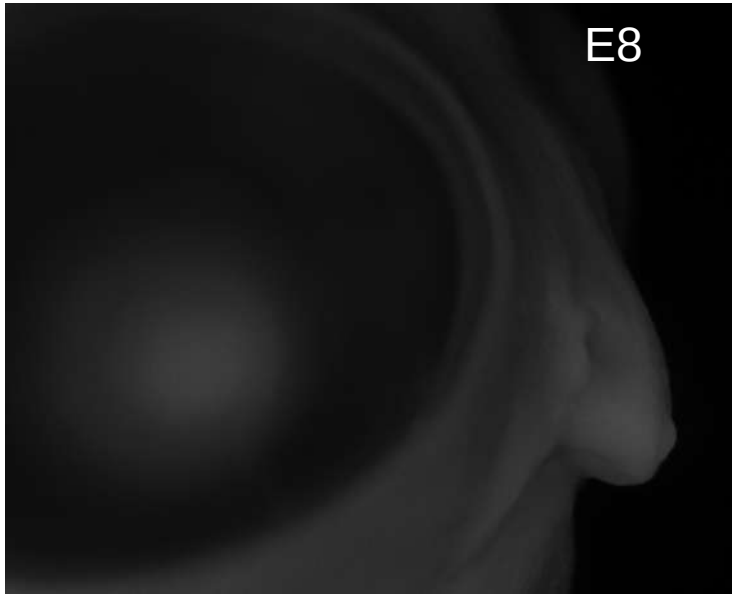
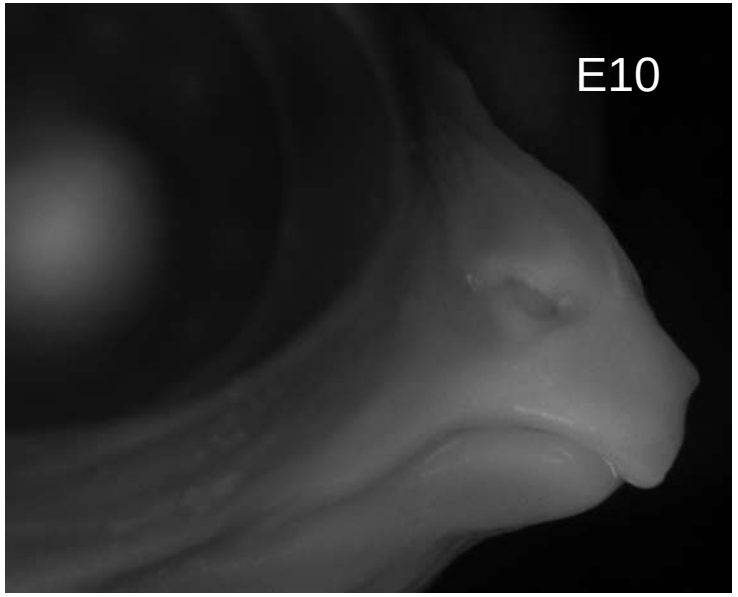
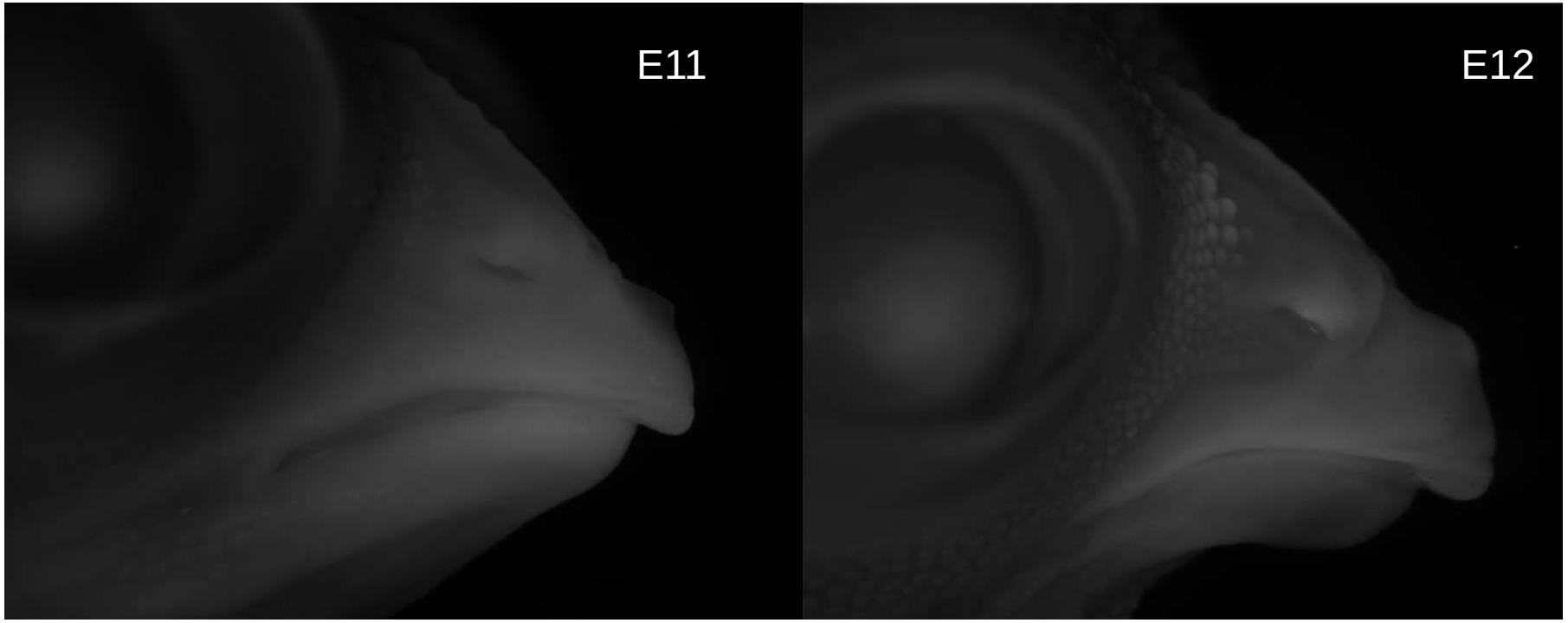






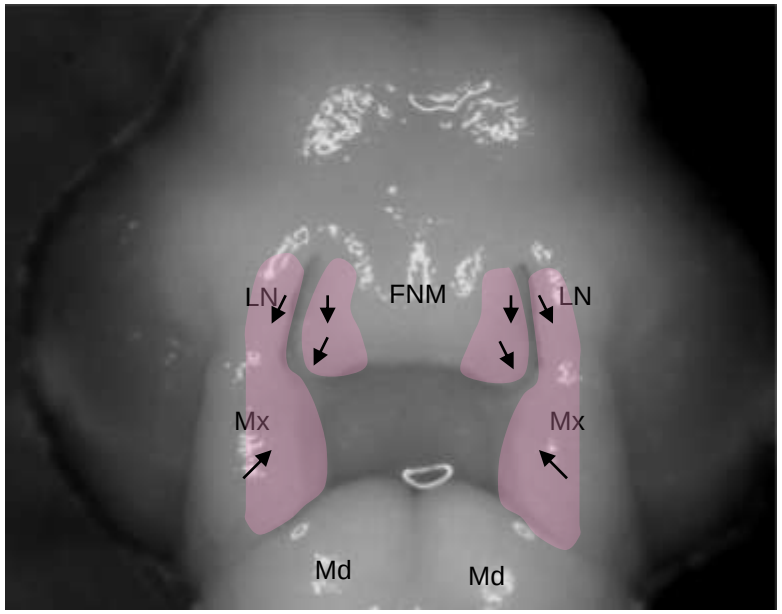
TREATED



TREATED

How could a reduction in bud expansion have originated a curved beak?

Different buds expand in different directions

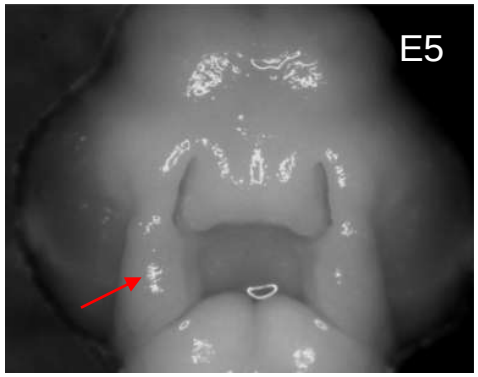


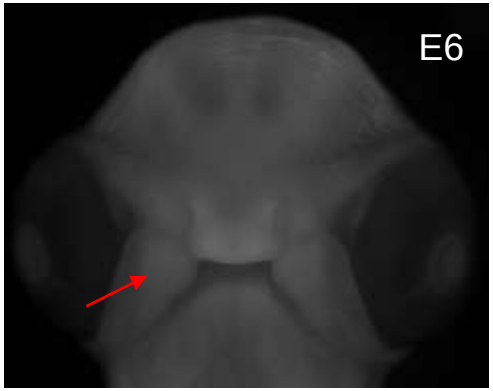
Black arrows indicate the direction of expansion of cell populations (McGonnell et al. 1998):

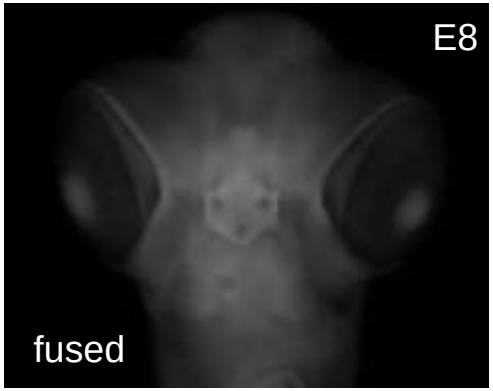
1. **FNM** and **LN** expand along a **mediolateral axis** (in the plane of the screen, towards the lower corners)
2. **Mx** expand along a **proximodistal axis** (towards the viewer out of the screen)

Beak growth

The FNM grows forward when is lifted by the maxillary buds

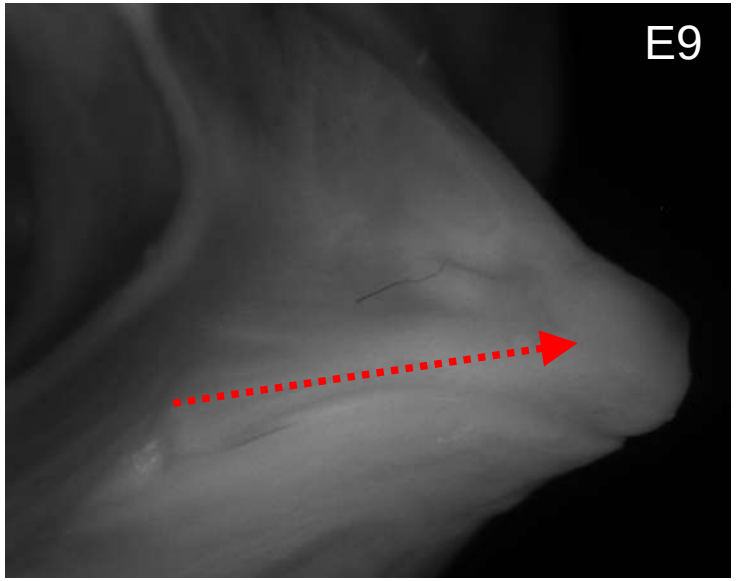




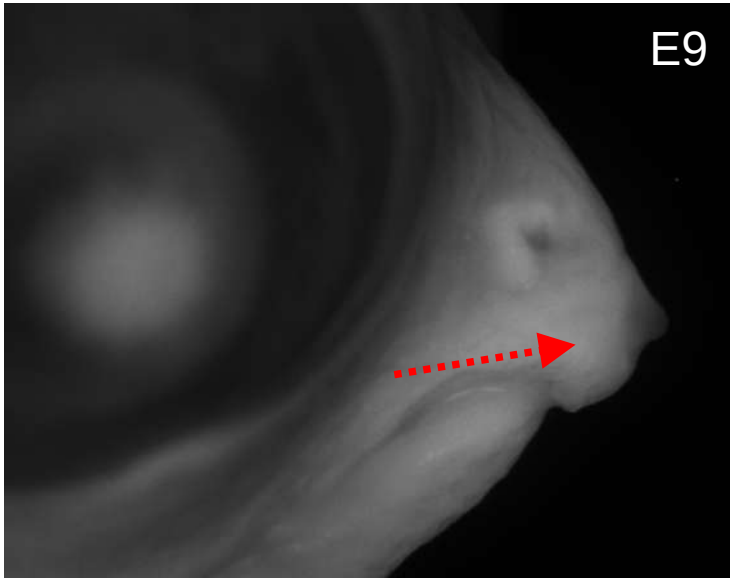


A curved beak

An induced curved beak in the chicken is not due to the alteration of the differentiation of skeletal tissue (cartilage or bone). It is instead the consequence of a reduction of bud expansion, so that the maxillary buds fail to lift the FNM

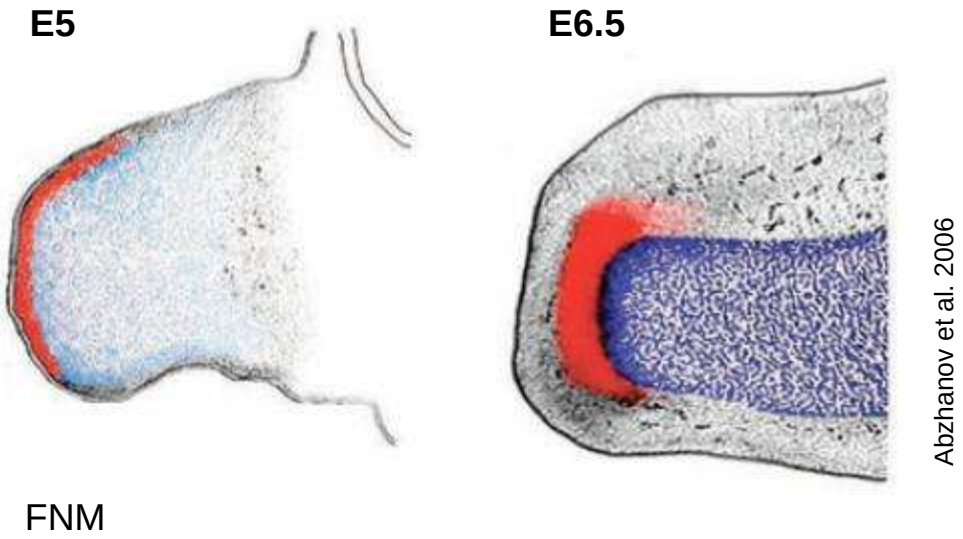


control



treated

Revisiting finches



FNM

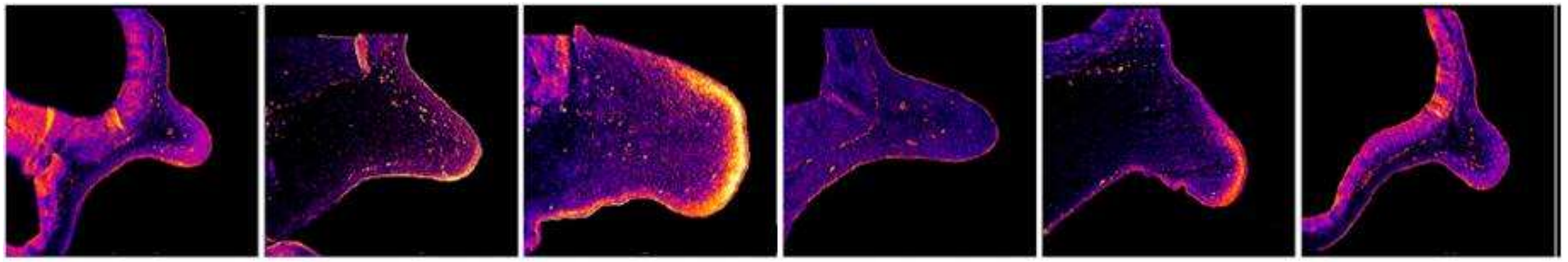
Bpm4

cartilage

chondrogenic mesenchyme

Geospiza magnirostris

Incipient forms already present at E5



Mallarino et al. 2011

Campàs et al. 2011

G. fuliginosa *G. fortis* *G. magnirostris* *G. scandens* *G. cornirostris* *G. difficilis*

A physicalist developmental model for the bird beak

A prospective model based not on developmental genes, but on the physics their products mobilize during beak morphogenesis (bud expansion and the physical interactions between buds)