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# ON THE CHARACTERIZATION OF EMBRYONIC CURVATURES USING BEZIER CURVES

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## INTRODUCTION

We study morphogenesis (shape changes) that occur during early embryo development. The shape of the early embryo is characterized by a series of well-defined curves. We study here the dorsal curvature of the early chick embryo, which is characterized by two distinct bends – cervical and thoracic flexures (Fig.1). We study the formation and deepening of these flexures starting at about Hamburger and Hamilton (HH) Stage 14 and following the development 9 or 10 hours of incubation later, when the embryo reaches HH Stage 15 or 16 [1].

The deepening of flexures is a significant morphogenetic event in the early embryo and usually they are described imprecisely and using adjectives. Reference [1], for instance, describes cervical flexure as "a broad curve". We need a mathematically precise way to quantify flexures and precise criteria that can be used to distinguish between normal and abnormal flexures. Prior research indicates that correct flexure formation is important for the development of major organs, including the heart [2]. In that study, we quantified the cervical flexure by fitting a circle and here we expand on those results, by fitting a parametric curve to the entire dorsal wall. We use Bezier curves, which are widely used in computer graphics and to precisely define font shapes, to describe the shape of the dorsal wall in early embryonic development.

## METHODS

The dorsal wall was "sampled" by selecting closely-spaced points using ImageJ. We used a total of 7 embryos ($n$=7). Measurements were taken at t=0 and at t=9h ($n$=2) or 10h ($n$=5). The cubic Bezier curve is a parametric curve where $t \in [0,1]$ is a parameter that varies from 0 (start of the curve) to 1 (end of the curve).

$$\mathbf{B}(t) = (1-t)^3\mathbf{a_0} + 3(1-t)^2 t\mathbf{a_1} + 3(1-t)t^2\mathbf{a_2} + t^3\boldsymbol{a_3} \qquad (1)$$

where $\mathbf{a_0}$, $\mathbf{a}_1$, $\mathbf{a}_2$, and $\mathbf{a_3}$ are the Bezier control points in 2D space. The line joining the Bezier control points is called the Bezier Polygon (Fig. 1). Unlike polynomial fits, wherein the coefficients do not convey insight about the shape of the curve, the Bezier control points, which are the curve parameters, are rich in meaning. To begin with, the first (last) control point is simply the first (last) point in the data set. The middle two control points ($\mathbf{a}_1$, $\mathbf{a}_2$) were fit using an optimization procedure in MATLAB. Also, the line joining the first two control points ($\mathbf{a_0}$, & $\mathbf{a_1}$) is tangent to the initial curve direction and the line joining the last two control points ($\mathbf{a_2}$, & $\mathbf{a_3}$) are tangent to the final curve direction. Note also that the movement of the middle control points from t=0 to t=9 or 10h quantifies the deepening of flexures and the movement of the first and last control points shows the increase in curve length (Fig. 1A,B). Bezier curves are also invariant under affine transformations.

The single cubic fit is unable to capture fully the sharpness of the thoracic flexure at the later time point (Fig. 1C). In this case, it is possible to fit a piecewise Bezier curve where the last control point of the first curve becomes the first control point of the second curve, i.e., $\mathbf{a}_3 = \mathbf{b}_0$ (Fig. 1C). Also, we impose a constraint for geometric continuity, i.e., $\mathbf{a}_2$, $\mathbf{b}_0$, and $\mathbf{b}_1$ are colinear. The ability to break up larger curves into smaller ones, each with its own control polygon, is a significant advantage to using Bezier curves to characterize complex shapes.

## RESULTS

The length of the curve, as measured by a numerical line integral using the selected data points, increases from 3.46±-0.27 mm at t=0 to 5.43±0.42 at t=9 or 10h. These values are similar to the Bezier curve lengths (data not shown).

Once a parametric curve is fit, we can measure the curvature using techniques from Differential Geometry. Fig. 1D shows the curvature for a single embryo (done in MATLAB and verified in Mathematica). Note that the composite curve fit is able to precisely capture the thoracic flexure, enabling us to calculate the curvature more accurately (Fig. 1C,D). For the composite fit, the curvature is very nearly straight for the first half of the curve (arrow in Fig. 1D), indicating that the cervical flexure portion can be approximated by a circle. For the embryo in Fig.1C, the radius for cervical flexure is about 2.5 mm ($\kappa \cong 0.4$, Fig. 1D), which is consistent with the results from our earlier study [2].

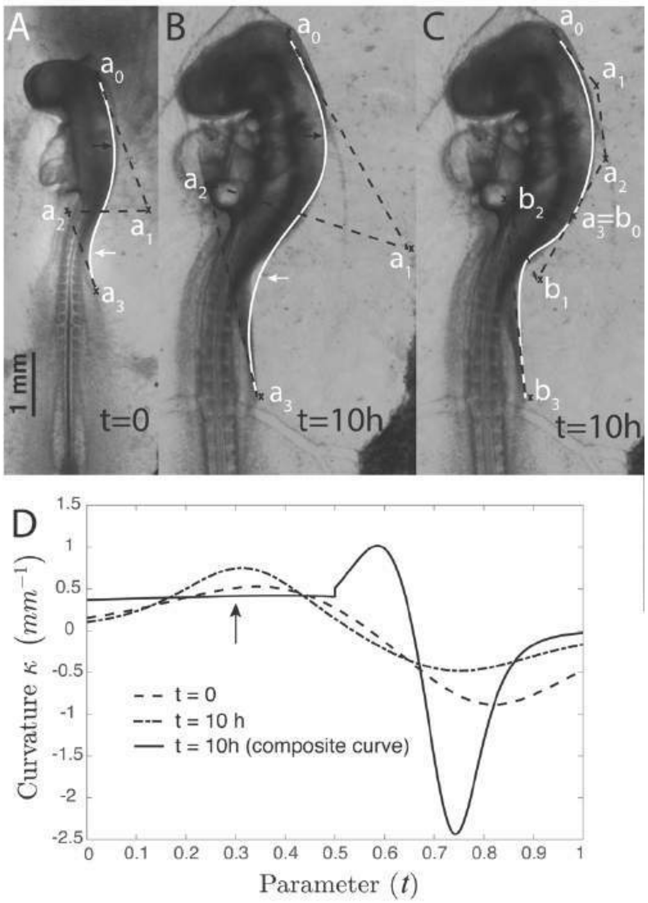


**Figure 1: Chick embryo at stage HH 14 (A) and 10 hours later (B, C), along with Bezier curve fits and curvatures (D). Black and white arrows in A & B indicate cervical and thoracic flexures, respectively; note the deepening of flexures at t=10h (B). A—C show the Bezier curve (white) and Bezier polygon (black dashed). In (A) & (B), $a_0$, $a_1$, $a_2$, $a_3$ are the four control points (marked by "x") of the single cubic Bezier curve. The middle control points move farther from the embryo (compare locations of $a_1$ & $a_2$ in B & C), indicating the deepening of flexures. The fit for the thoracic flexure is poor in (B), a situation remedied by the composite Bezier curve fit in (C). Curvatures for the curves shown in (A—C) are reported in (D). The curvature information can be used to develop finite element models (Fig. 2B) that give insight into the mechanisms driving flexure formation. The reader is cautioned not to confuse the parameter *t* (*italic*; used in the horizontal axis in D) with the time stamp t used to denote progression of embryo development.**

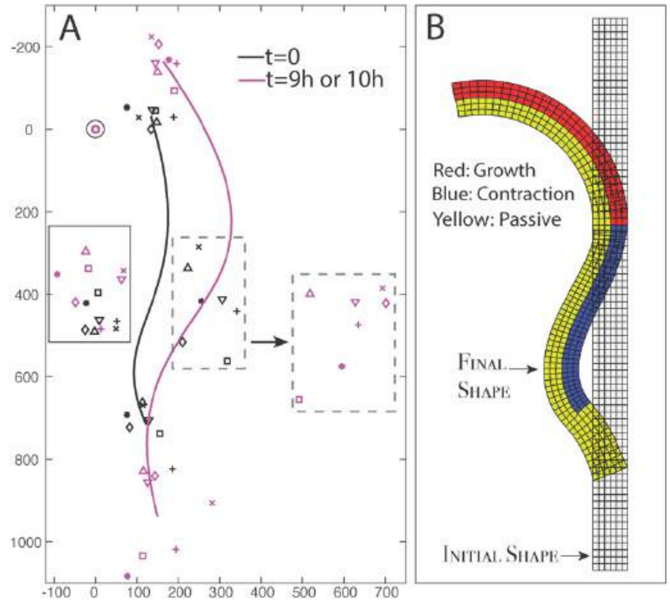


**Figure 2: (A) Bezier control points "constellation" from curve fits on 7 embryos, each shown by a different marker (+,* etc.). Pixel dimensions are used (225.696 pixels = 1 mm). An affine transformation (translation) is carried out with the embryo eye as the origin (denoted by the bull's eye symbol at 0,0). Black markers denote t=0 while purple markers denote t=9 or 10h. Solid curves show the average Bezier curve fit based on the Bezier control points presented. Note that the second control points (enclosed in the dashed boxes; again, black and purple symbols denote t=0 and t=9 or 10h respectively) move farther (black arrow), indicating a deepening of cervical flexure. There is not a similarly large shift in the third control point location (solid box), which corresponds to the thoracic flexure; hence we use a composite curve to better capture the thoracic flexure (Fig. 1C). (B) Results from a finite element model (done in ABAQUS) of the dorsal wall with growth (contraction) specified in regions of positive (negative) curvature. Undeformed (black) and deformed (color) shapes are shown.**

## DISCUSSION

Cubic Bezier curves have a number of elegant properties which make them ideal to quantify shape changes during morphogenesis. We found that a single cubic Bezier curve captures the overall features of the dorsal wall. The control points (Fig. 2) and the control polygon (Fig. 1) are useful tools to broadly characterize shapes. Composite curves can be used to fit cubic curves for smaller sections when more precision is desired (Fig. 2C). Tools from differential geometry such as curvature, arc length, and velocity of curves can be used to develop finite element models and investigate mechanisms driving morphogenesis. Bezier curves can be used to compare curvatures across different time points, control vs. perturbation conditions, and also for comparing embryo shapes from different species at the same developmental period.

## ACKNOWLEDGEMENTS

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## REFERENCES

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[2] Ramasubramanian, A et al., *J Biomech Eng*., 141:1-12, 2019.