

# Anatomical complexity is acquired at an exponential rate during mouse embryonic development

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## Abstract

Embryos become more complex, in terms of anatomy and of cell differentiation, as they develop. A metaphor for differentiation is Waddington's 'epigenetic landscape', in which cells move along channels that bifurcate repeatedly, each branch leading to the production of a distinct state or structure. Choices at the model's bifurcations are controlled by cells' internal states combined with intercellular signals, in more complex combinations as the complexity of the embryo rises. This successive bifurcation model predicts that the number of distinct entities embryos should rise exponentially as it develops. This study examines existing data on the development of the mouse, for which a detailed, staged tissue ontology is available, and tracks the rise in distinct entities over time. In the whole embryo, in the developing kidney and in the developing reproductive systems (the only sub-systems for which sufficiently detailed ontologies are available), the number of cell types rises exponentially during the patterning phase of development ( $R$ -squared= 0.945 to 0.985). These results are compatible with (but do not prove) Waddington's idea that, in general, already-gained complexity is used to generate further complexity and new choices in embryonic life.

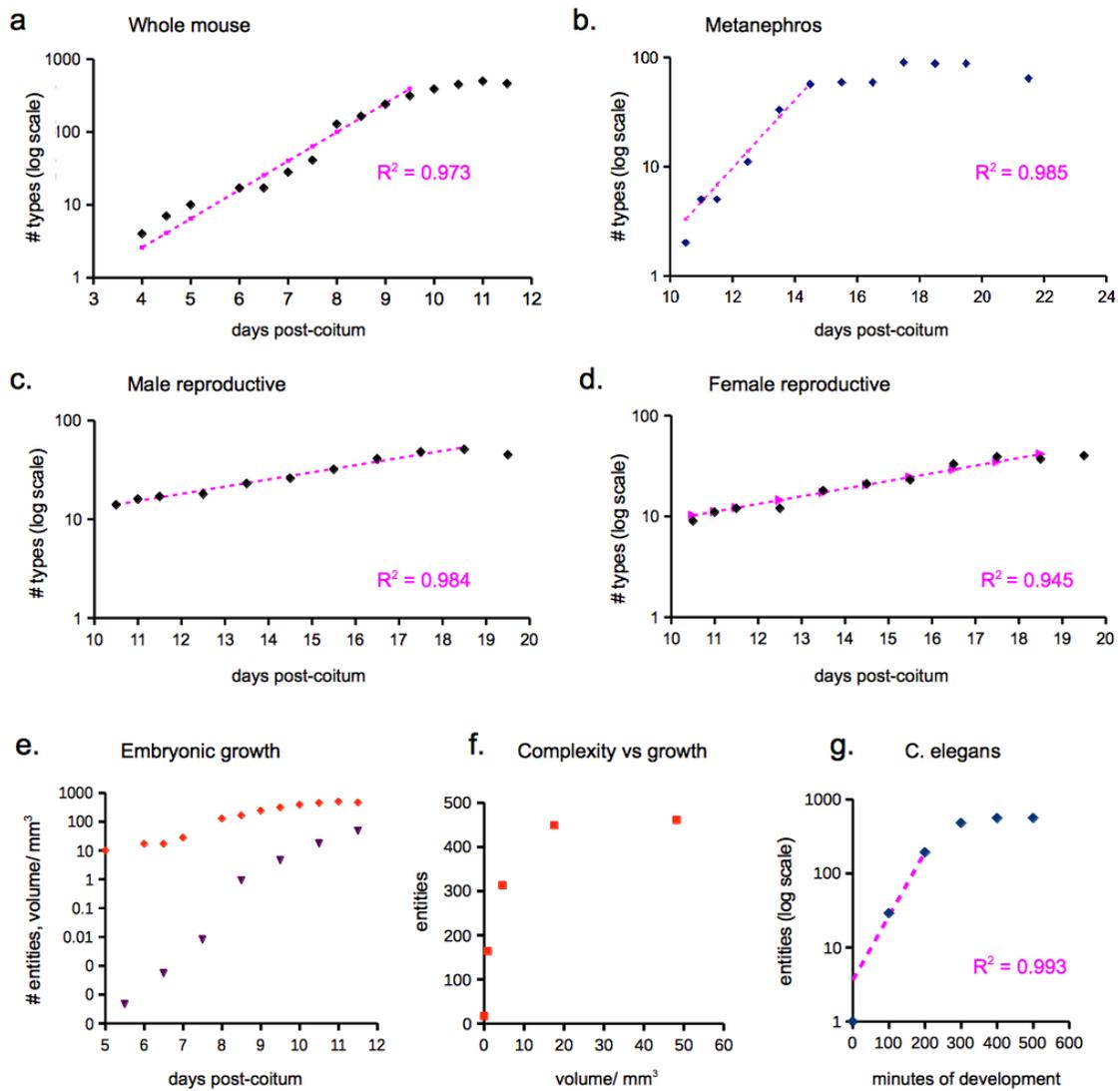
## Introduction

Adult bodies differ from those of early embryos in two main ways: size and complexity. Size is easy to define and measure, and many mechanisms of size control, from the scale of cells to the scale of whole organisms, have been discovered [1] [2] [3] [4], although there is undoubtedly much still to learn. Complexity, on the other hand, is harder to define and measure. C.H. Waddington was one of the earliest embryologists to consider rising complexity: he used the rising number of differentiated states in a tissue as a proxy for complexity [5], regarding it to be achieved by changes in gene expression caused by a combination of 'segregation' (of cytoplasmic determinants in cell division), 'evocation' (inductive signalling between dissimilar neighbours to create new cell types at the boundary, as discovered by Spemann and Mangold [6], and 'field action' (differentiation in response to a morphogenetic gradient). He also introduced the famous image of differentiation as a river-delta-like branching of channels as development 'flows' from zygotic simplicity to adult complexity [7]. Later work added more detail to the idea of field action [8] and used computer models to explore further the idea that interacting cells can generate differentiated states, from the same genome, that are inaccessible to cells in isolation [9].

The Waddington view of cell differentiation as a series of bifurcating channels ('creodes', to use his term) is a striking image, and it carries an interesting implication: if the events that drive this rise in complexity occur at a constant rate, the number of cell types would be expected to rise exponentially, as one branch becomes two, which become 4, which become 8 and so on. In principle, this is testable from data on real embryos. It happens that, for other purposes, a detailed list of embryonic components has been made at different times of mouse development. This resource allows a simple test to be made about whether complexity, in terms of cell types, really does rise exponentially. The results show that, for the embryonic period, it does: the rise flattens to a plateau during foetal life.

## Objective

To analyse published ontologies [10] of mouse embryonic components to test whether the rise of complexity in developing embryos is exponential with respect to time, during embryonic patterning.



a

### Figure Legend

**Figure 1. The rise of complexity is exponential during patterning of the mouse embryo as a whole and during patterning of its components.**

(A) Shows a log-linear plot of the number of distinct entities in the EMAGE mouse ontology with respect to time (days post-coitum): the magenta line depicts the best-fit exponential for the embryonic patterning period, from 4 to 9.5 days.

(B) Shows the same type of plot for the entities in the GUDMAP ontology of the metanephros (permanent kidney).

(C & D) Show the same type of plot for the entities in the GUDMAP ontology of the male and female reproductive systems. In all cases there is an exponential rise during the period of patterning (reproductive development continues after this period, but the published ontologies do not, ceasing at Theiller stage 28).

(E) Depicts the rate of increase in entities (red) and the increase in embryo volume (a proxy for cell number: blue) against time, while (F) plots number of entities against volume: clearly rise in entities does not simply shadow rise in cell number: if they did, (F) would show a straight line.

(G) Shows a plot similar to (A) but for *C. elegans*: see the ‘conjectures’ section for comment on this. In all cases, correlation coefficients shown in magenta are from the period of development indicated by the magenta dotted line.

The organisms and organ systems chosen for this analysis were those for which detailed stage-by-stage ontologies have been constructed. The ontological data sources and Theiller stage (TS) ranges were as follows:

\* whole mouse: ontologies TS 4 - TS 20 downloaded from <http://www.emouseatlas.org/Databases/Anatomy/new/>

\* mouse reproductive system: ontologies TS 17 - TS 28 downloaded from [www.gudmap.org](http://www.gudmap.org)

\* mouse metanephros: ontologies TS 17 - TS 28 downloaded from [www.gudmap.org](http://www.gudmap.org)

\* *C. elegans*: ontology from figure 3 of [19].

The ontologies were printed out for each stage, as a hierarchical tree, and the list of entries for each stage was counted manually. Where terms at the finest level of detail (e.g. *Sertoli cell*, *peritubular myoid cell*, etc., in the testis) included a complete list of children of a less detailed term (in this case, *primary sex cord*), the higher-level term was ignored to avoid double-counting.

The counts were entered into a spreadsheet (LibreOffice Calc) column, the age of the embryo corresponding to the ontology in question was entered into an adjacent column, and the log of the number of cell types was calculated in a third column. These columns were used to plot the graphs in figure 1. The graphs were used to identify the point where the curve obviously flattens, and data points before that were exported into PRISM for exponential regression analysis; for each data set, the best-fit line from this analysis was then entered into the spreadsheet and also plotted on the relevant graph in figure 1.

Data on mouse embryo volume, used as the best-available proxy for cell number, came from two sources: direct measurements of embryo volume from 5.5 to 7.5 days post-coitum were obtained from the normal embryo data in [20]. Measures of embryo volume from 8.5 to 11.5 days were obtained from data on crown-rump length and diameter in [21], by treating the embryo as a cylinder and multiplying cross-sectional area by length. While this may have introduced errors, possibly up to a factor of two, these would be insignificant compared to the many-orders-of-magnitude differences between volume growth rates and complexity growth rates plotted in figure 1E. **Results &**

## Discussion

### The mouse embryo as a whole

The EMAGE ontology of mouse development has been constructed in an on-going project to list all components of the developing embryo, foetus and adult [11]. Annotation of the embryonic period (from fertilisation to the laying down of organ primordia, days 0–10) can be considered complete. Annotation of the foetal period is patchy: the ‘whole body’ information is there up to about day 18 [12] and some individual organ systems have been annotated thoroughly for other projects such as the GUDMAP urogenital project ([www.gudmap.org](http://www.gudmap.org)), but the majority of organs still await this highly detailed treatment. The EMAGE whole embryo can therefore be considered as a reliable source of information on total numbers of cell types only during the embryonic period, and for this reason only the ontologies up to day 11.5 (Theiler stage TS19) [13], a little beyond the embryonic period, were included in the analysis.

The result of plotting the number of ontological entities in the embryo against developmental time is shown in figure 1A. From 4 days (the blastocyst- the beginning of differentiation [14] until 10 days, the number of entities rises, following a line that is very close to exponential (the best-fit exponential line fits with  $R^2 = 0.97$ ). After this point, the curve flattens (see below).

### The metanephros

The rudiment of the metanephros (permanent kidney) appears in mouse at E10 and is patterned over the next 5 days or so: almost all cell types are present by E15 [15]. A detailed ontology of the process has been constructed for the GUDMAP project [16]. A plot of the number of entities listed in this ontology, against developmental time, is shown in figure 1B. From the beginning of metanephros development until E15, the rise in cell types is remarkably close to exponential ( $R^2 = 0.99$ ). Thereafter, as patterning gives

way to maturation, the trend flattens considerably and even begins to fall as entities that are not present in the mature organ disappear.

### **The reproductive system**

Development of the reproductive system begins at about E10.5 and continues until puberty, in the case of males, and arguably until menopause in the case of females (the menstrual cycle and pregnancy involve developmental events). The accurately timed GUDMAP ontology for the reproductive system extends to TS 28 (about 19.5 days). Plots of the number of entities in the ontologies for the male and female reproductive systems against time are shown in figures 1C, D: both show approximately exponential rises ( $R^2=0.98, 0.95$  respectively) with very similar slopes. The male shows a fall in cell types in the last data point, when the patterning phase has given way to maturation.

### **Complexity does not simply track growth**

One possible explanation for an exponential increase in anatomical entities might be an underlying exponential increase in cell number. Direct counts of cell numbers do not seem to have been published for mouse embryos beyond the earliest stages, but volume is a reasonably proxy for total cell number given that most mammalian cells are of similar size. It can be seen from figure 1E that entity number grows much less steeply than volume, with respect to time, and from figure 1F that the correlation between entity number and volume is very far from linear. Complexity is therefore not a direct, linear effect of cell proliferation in this system.

All 4 ontologies examined indicate an exponential rise in complexity in the early period of their system's development, a period that corresponds well with that of patterning. The metanephros and whole-embryo data show a clear transition to a much flatter rise and eventual small fall in complexity during maturation (the same may be true of the reproductive system but the available ontologies end too soon to test this). The apparent timing of this flattening in the whole-embryo data may be premature, an artefact of the incomplete annotation in the foetal period mentioned above: a fully annotated whole embryo would of course contain information on all of its developing organs at the same level of detail as the three organ systems described here.

## **Conclusions**

The data indicate that the number of distinct entities in the embryo, as defined by peer-reviewed and widely used ontological descriptions, increases exponentially with time during the period of patterning. This observation is compatible with Waddington's view of differentiation being a series of bifurcations in an epigenetic landscape, particularly if the events that drive these bifurcations- any combination of cytoplasmic segregation, induction and response to morphogenetic gradients [17] happen to cells at a constant average rate.

## **Limitations**

There is one potential weakness in the study design. Waddington focused on cells, while the ontologies on which this work is based are lists of anatomical entities rather than explicitly of cell types. In many cases the entities listed *are* cell types (see Suppl. Fig. S1) and in most others the correspondence between an anatomical entity (e.g., 'segment 1 of proximal tubule') clearly corresponds to a cell type (in this case, a segment 1 proximal tubule cell). In a few cases, however, it is possible that the cells corresponding to distinct anatomical entities (e.g., capillary endothelium within different organs) might actually be in exactly the same state of differentiation. There is therefore some risk that using these ontologies as a proxy for counts of differentiated states will overestimate the number of states. Inspection of the entities within the ontologies suggests that the number of cells that could be the same in different tissues (blood capillaries, neurons, etc.) represent less than 10% of all entities and so is not a significant risk to the analysis. Also, the trend in biology in the molecular era is for one-time cell types to be split into multiple sub-types as gene and protein expression data, and sometimes physiological data, show that cells that once appeared to be the same are not the same. An example is provided by T cells, which are now known to be of at least 7 types, with the possibility of further splitting being done in future [18]. It therefore seems unlikely that the use of

these ontologies has overestimated cell types in any serious way.

Whether or not the exponential rise in complexity described here results from a mechanism like Waddington's or has another cause, it would be interesting to test whether it is seen during the period of patterning in phylogenetically divergent animals such as arthropods or molluscs, and might therefore be regarded as a 'universal' feature of animal development. At the suggestion of a reviewer of the first draft of this manuscript, I have examined the rise in complexity in *Caenorhabditis elegans*, one invertebrate for which a rich embryonic ontology is available. A plot of *C. elegans* entities against developmental time, shown in figure 1G, again shows an initial exponential increase in entities with respect to time, followed by a flattening of the curve. It is important to note, though, that the criteria for ontological entities in *C. elegans* and mouse are different: *C. elegans* is so simple, and its development is so reproducible, that each cell in *C. elegans* is identified individually by position, even if it is otherwise similar to another cell. In the case of *C. elegans*, the exponential increase can therefore be explained directly from cell division. In mouse, entities are generally multicellular and, as shown in figures 1E, F, entities increase much more slowly than does embryo volume (a proxy for cell number). Proper comparison across phyla awaits the construction of invertebrate ontologies that use similar rules to those used for mouse and which would therefore be more likely to be made for larger invertebrates, such as octopus.

## Additional Information

### Methods

The organisms and organ systems chosen for this analysis were those for which detailed stage-by-stage ontologies have been constructed. The ontological data sources and Theiller stage (TS) ranges were as follows:

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### Supplementary Material

Please see <https://sciencematters.io/articles/201604000005>.

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## Ethics Statement

Not applicable.

## Citations

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