THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF ANTHROPOLOGY

ASYNCHRONY OF DEVELOPMENTAL MARKERS IN EMBRYONIC MICE: THE NEED FOR A NEW SYSTEM

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ABSTRACT

Mice are the primary model organisms used to study mammalian embryological development. Consequently, researchers have intricately described their developmental features and sequence, and have used these traits to establish biological staging systems on the basis of age-specific developmental markers (e.g. number of somites, crown-rump length, limb bud development, craniofacial morphology). However, little research has been done to examine the magnitude of variation present within mice that are of the same chronological age (number of days of development post conception). Indeed, individuals within litters are known to be of different ages as egg fertilization takes place individually so the timing of each conception is unique. As proof of this, genetically invariant littermates often show signs of being of different developmental ages, an observation that means that the conception of the embryos occurred at different times and that each embryo may follow a different growth trajectory and potentially grow at a different rate. Due to these differences, a different age may be estimated for an embryo depending upon which morphological element and corresponding staging system is used. In this study, crown-rump length, digit development, and whisker and facial follicle presence were recorded for mouse embryos betnween embryonic days 12 through 16 (E12 - E16). These data were used to characterize the structure of variation in these traits during this embryonic period and to determine whether these patterns reveal coordinated or asynchronous development across systems. My results indicate wide variation across traits and developmental timing. Significant overlap occurs in the younger ages (i.e. E12 - E14) for crown-rump length and limb bud maturation, while the older embryos (i.e. E15 -16) exhibit increased variation within the measured traits.

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Chapter 1

Introduction to Mouse Models and Embryonic Staging Systems

Developmental biologists often characterize mammalian development as a singular and physiologically unified progression toward a preset outcome varying only moderately between the individuals of a particular species. However, variation exists within nearly all biological processes within and across specimens[4,9,12,13]. Systems of the body develop at different rates according to biological timetables and, although all systems communicate in some sense throughout all stages of development, the development of these systems is not always uniform[1,7]. During prenatal development specifically, growth occurs rapidly as cells differentiate to establish various tissues and organs. Developmental changes that occur during this concealed prenatal period are difficult to observe and therefore, many of the processes are less well studied.

Researchers commonly utilize animal models to better understand prenatal growth mechanisms. Although zebra fish and chick are often used in biological research, mouse models are commonly used to study human development and pathogen response because of their relatively close phylogenetic relationship with other mammals, including humans. Many animal model studies have attempted to characterize mammalian growth as a process with distinct stages[2,8,12,15,17]. In utero, mouse littermates are fertilized at nearly the same time and, therefore, are considered to be of the same chronological age. However, pups within a single litter often exhibit different levels of biological maturity, or show characteristics of different developmental stages[2,16], as can be seen in Figure 1. Recognition of this fact has led researchers to attempt to

establish linear staging systems based on various physiological features used as markers to assess biological age and developmental timing.

Figure 1. Developmental Differences Between Genetically Identical Littermates.

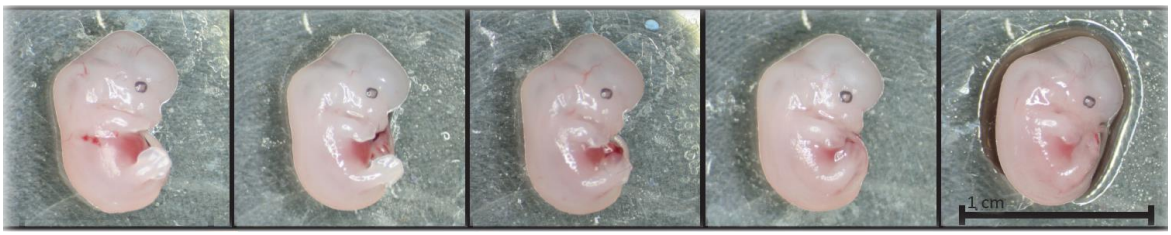


Figure 1. A litter of C57BL/6J mice at embryonic day 12 (E12), demonstrating the differences in size and maturity within a single litter. Not only are the embryos different in size, the level of maturation of their heads, their limbs, and their embryonic tails vary. *Photograph by Kevin Flaherty.*

One reason that it is difficult to precisely age embryonic mice comes from the inability to observe the moment of conception in mammals. In overnight breedings, fertilization can only be recorded as occurring during an estimated span of time. In addition, each embryo is fertilized individually in the uterus resulting in variation in conception timing between littermates. Although there is no adequate method for eliminating these disparities, it is important for researchers to be aware of known confounding factors when comparing developmental data from prenatal specimens that are assigned to the same embryonic age.

Existing Mouse Staging Systems & The Need for New Methods

Karl Theiler outlines at length the prenatal development of the mouse embryo in his 1989 book on the subject[15]. He categorizes embryonic development into 26 stages prior to birth. At each stage, he describes significant growth events that serve to distinguish the stage from others and provides details about the corresponding development of internal systems of the embryo. Although he identifies changes to both internal and external structures, developmental

researchers often only use externally visible markers to estimate biological age. One of the external characteristics measured consistently at each Theiler stage is crown-rump length. Theiler's results show little, if any, overlap between stages for crown-rump lengths beginning at Theiler stage 17 (embryonic day 10.5 or E10.5) until birth (19 days after conception; labeled P0) and only very little overlap observed in lower stages. With known biological variation present between any two specimens, it is unlikely that no overlap exists in these measurements between embryos, especially between non-littermates. Likewise, each stage describes simultaneous development occurring across multiple anatomical systems, further complicating these proposed stages. With the intent to characterize holistic development of the mouse embryo, the Theiler system successfully chronicles general progression of prenatal mouse development, but individual-specific variation is ignored. The finely detailed descriptions leave very little room for deviation from stages, making them difficult to use for estimating developmental age of an individual embryo.

Boehm et al. (2011) used 2D morphometric methods to analyze the mouse hind limb bud using curvature graphs to track the continuous yet stereotypical shape changes of the limb occurring throughout embryonic development. This study focused on developing an accessible and replicable staging system for mice characterized by generalized limb bud shape change[2]. The authors admit that, although their research depicts and describes morphological changes according to chronological time since conception, this system cannot account for the variation between individuals and that not all mouse embryos will reach the same developmental point within a given amount of chronological time.

The central problem revealed by the consideration of these and other staging methods is that, although each of these schemes is valid, they may provide inconsistent and/or conflicting

information about the developmental stage of a single organism. If anatomical features do not align uniformly within individuals during prenatal development, some systems may categorize an embryo as corresponding with a particular developmental stage while others may classify the individual as belonging to a more or less mature phase in terms of developmental anatomy. This makes comparisons among individuals difficult and suggests that one staging system cannot, necessarily, reliably speak for the entire growth progression of an embryo. Moreover, care must be taken if researchers are comparing organisms but using separate staging systems.

Chapter 2

Research Questions and Goals

Mice are commonly used as models for human genetic diseases due to their phylogenetic affinity, anatomical correspondence, and analogous phenotypic traits and disease responses[15]. Correspondence of overall structure between divergent mammalian species demonstrates that a mechanism for conserving vertebrate morphology operates across taxa[10,13]. Many genes are conserved across diverse mammalian lineages, and many of their accompanying functions are likely conserved as well[10]. The use of mouse models provides the capability of studying early stages in development, which cannot be studied in humans due to obvious ethical restrictions. For these and other reasons (e.g., relatively short generation time, similarity in genomes, etc.), mouse models are often used in the analysis of embryological development. While existing literature of developmental markers has focused on the analysis of individual features in the formulation of staging systems[2,8,12,15,17], studies using mice and multiple staging methods might

eventually provide valuable information about human developmental timing and growth variation.

There is a critical need to evaluate the variation within and across mice during embryonic growth periods. Current and future developmental biology research relies on understanding the physiological processes occurring during prenatal stages of development. Because of the variation observed in human postnatal development, at both the level of the individual and the population, it is likely that the processes that occur in utero are not uniformly coordinated throughout prenatal development and that the different anatomical systems of a single embryo develop according to independent growth trajectories. If the currently proposed staging systems used to estimate biological age are not synchronous in these contexts, as assumed, new techniques will need to be implemented to understand holistic development.

Here I evaluate the developmental staging of embryos of known chronological age using several standard staging system markers. My goal is to determine whether staging systems based on different aspects of the developing embryo provide complementary or disparate information about the timing of development of embryos. This study also seeks to analyze and quantify the degree of asynchrony between growth progressions of individual features in the hopes of inspiring a new, more integrated approach to staging embryos. To do this, I design a provisional limb bud staging system similar to those developed by Theiler[15] (1989) and Wanek[17] (1989). I then compare results using this system with additional morphological features characterizing prenatal growth, such as crown-rump length and whisker row and facial follicle appearance.

Although a new staging system is not established here, existing staging systems are critically evaluated. My data will contribute to the understanding of development and to the establishment of improved staging systems. Such techniques would create a more accurate view

of embryonic developmental timing and allow researchers to address specific questions pertaining to individual variation in developmental timing during this critical growth period.

Materials and Methods

Breeding

The sample for this study consists of 298 (n=298) prenatal C57BL6/J-strain mice, with mice representing each day of embryonic development from E12 to E16 (Table 1). Mice were bred at the Pennsylvania State University. Timed overnight matings were used to produce embryonic mouse litters. Pregnant mice were euthanized at 9:00 a.m. at each designated day of embryonic development (i.e. E1 is one day after conception). Embryos were extracted, fixed in 4% paraformaldehyde (PFA) for 24 hours, and preserved in 0.01% PBS/Sodium azide solution. Each specimen was scored for each of four external developmental markers: crown-rump length, digit separation, whisker presence, and follicle presence. All litters were produced, sacrificed, and processed according to animal welfare guidelines approved by the Pennsylvania State University Animal Care and Use Committees (IACUC) following an approved protocol (IACUC#: 46558; IBC#: 46590).

Table 1 Total Sample Sizes for Each Developmental Marker Observed at Each Age

Table 1. Numbers of specimen data recorded for each trait may vary due to damage caused by harvesting and preserving embryos.

Embryonic Age	**Sample Size**				
	total	**crown-rump length**	**digit separation**	**whisker presence**	**follicle presence**
E12	69	30	30	41	41
E13	54	42	42	48	48
E14	49	39	39	44	44
E15	47	39	39	44	44
E16	79	41	41	59	59
All Ages	**298**	**191**	**191**	**236**	**236**

Crown-Rump Length

Monochrome 12-bit photographs of each mouse were captured using a QImaging Retiga 2000R camera. Image files were then imported into the computer program ImageJ[11]. Using the straight segmenting tool, 4 mm on the visible ruler in each photograph was highlighted. Selecting the "set measurement" option, the number of pixels within this length was then scaled to 4 units (mm). Next, the segmenting tool was used to select and measure the maximum distance from the crown of the embryo to the rump (Figure 2). This measurement was recorded for each embryo on two separate occasions to determine intraobserver error. An average of the two measures was used in analysis to improve measurement accuracy.

Figure 2 Example of Crown-Rump Measurement

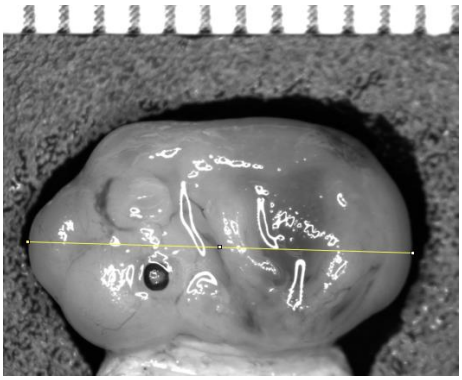


Figure 2 demonstrates the measuring of crown-rump length of an embryo using ImageJ. A mm ruler, shown here, is used to scale pixels. The longest distance across the body of the embryo is then recorded.

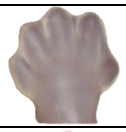
Although measures were taken to reduce bias and error, inaccurate measurements could potentially have been recorded. Likewise, mistakes in entering measurements into the digital data set could also contribute to confounding error in the results. I was aware of these possibilities before beginning data collection and prioritized accuracy and precision over speed of collection.

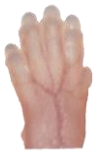
Digit Separation

Because of the chronological age range examined in this research, digit separation was selected as one of my investigated traits. The forelimb bud first appears at embryonic day 9 ½ (E9.5) with finger development beginning on E12[15]. Using a Zeiss SteREO Discovery.V8 microscope and spatula probe, each limb bud was individually examined and scored based on a developmental scoring system developed by Kevin Flaherty, a Ph.D candidate in the Richtsmeier Lab, and myself (Table 2). The digit scoring system was employed during this study to quickly and efficiently categorize a large number of limb buds according to their degree of development. The scale was loosely based on the efforts of Theiler[15] (1989) and Wanek[17] (1989), but with the intent to depict simple, yet significant, structural changes occurring during limb bud formation at these ages. Ultimately, the system was collapsed into five categories, or grades, that characterize the progression of limb development (Table 2).

Table 2 Scoring System for Digit Development

Table 2. Description of the five stages used to characterize digit separation in the fore and hind limb buds with photographic examples for each score.

0 = ROUND		Limb bud is a continuous, round shape, with no visible indentations marking the spaces between digits
1 = LINEAR/ POLYGONAL		Limb bud is no longer a continuous, round shape. Flattened edges exist between digits, still with no visible indentations
2 = INDENT		Indentations are clearly present between digits with one or more angles **>90°** formed by connection of 2 digits
3 = SEPARATE		Indentations are clearly present between digits with all angles formed by connection of 2 digits **≤90°**

4 = CONTOUR		Digits have distinct contour on distal portion that delineates the location of the finger pads and nail bed

In order to be placed into any of the five categories, all inter-digit tissue shape must fit the listed description of the numbered stage. That is, if one segment of the limb bud appeared to retain vestigial characteristics of a previous stage, that limb bud was recorded as being of the more immature of the two stages. Some embryos had missing or damaged limb buds and these occurrences were not scored for analysis. Ultimately, right hind limb digit scores were selected for analyses, as this group was the least affected by damage. In addition, hind limbs generally trail the forelimbs in development[12,15], and therefore our hind limb data include more variation at E12 than was observed in the forelimb digits.

Because of the sheer number of embryos examined for this trait, errors in classification are possible. Care was taken to remain consistent throughout the duration of the project using the listed definitions for each stage. Data entry error is also possible, but efforts were taken to double check database numbers and to look for unusual outliers.

Whisker and Facial Follicle Presence/Absence

Figure 3. Facial Follicle Location

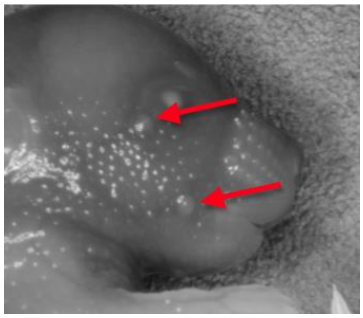


Figure 3 illustrates the placement of the two notable facial follicles on an E16 mouse embryo.

As with limb bud observation, whisker and facial follicle presence were examined using a Zeiss SteREO Discovery.V8 microscope and probing instrument. Using the probes, the head of each embryo was inspected for follicle development. If any whisker formation appeared to have occurred on the snout, presence was recorded for the embryo. Likewise, the two facial follicles, the most anterior located near the mouth, the more posterior located near the eye (Figure 3) were recorded as being present or absent. The follicles seem closely linked and to appear jointly during development.

In the younger ages E12 and E13, whisker and follicle development was admittedly difficult to observe. Therefore, misclassifications of presence or absence are possible. Caution was taken when recording this data to minimize bias.

Data Analysis

Analysis of the data was performed using the program R[14]. Inter-trait variation was analyzed through the creation of simple graphs and plots rendered from the data set. For binary traits, such as whisker and facial follicle presence, tables were created to evaluate variation in the timing of appearance. A scatter plot was created to examine the inter-trait covariance of the right hind limb digit scores and crown-rump.

Chapter 3

Results

Intra-trait Variation

Crown-Rump Length

Crown-rump length measurements at each chronological age were analyzed using the coding software, R[14]. Figure 4 shows the scatter plot distribution for crown-rump length averages for each specimen. The data include ages E12 - E16 and are sorted into each labeled age group by color. Figure 5 illustrates the variation in crown-rump length of specimens at each age. The bars of the histogram reflect the density of the sample size for each age group within each crown-rump length interval marked on the x-axis. An overlap in crown-rump length is visible between adjacent ages in Figure 5, including individuals with chronological ages up to 48 hours apart in either direction. More overlap was observed across the younger specimens (i.e., E12 - E14) while within-age variation was less for the younger age groups. Variation in crown-rump length values increased dramatically in ages E15 and E16.

Figure 4. Scatter Plot Distribution of Crown-Rump Lengths

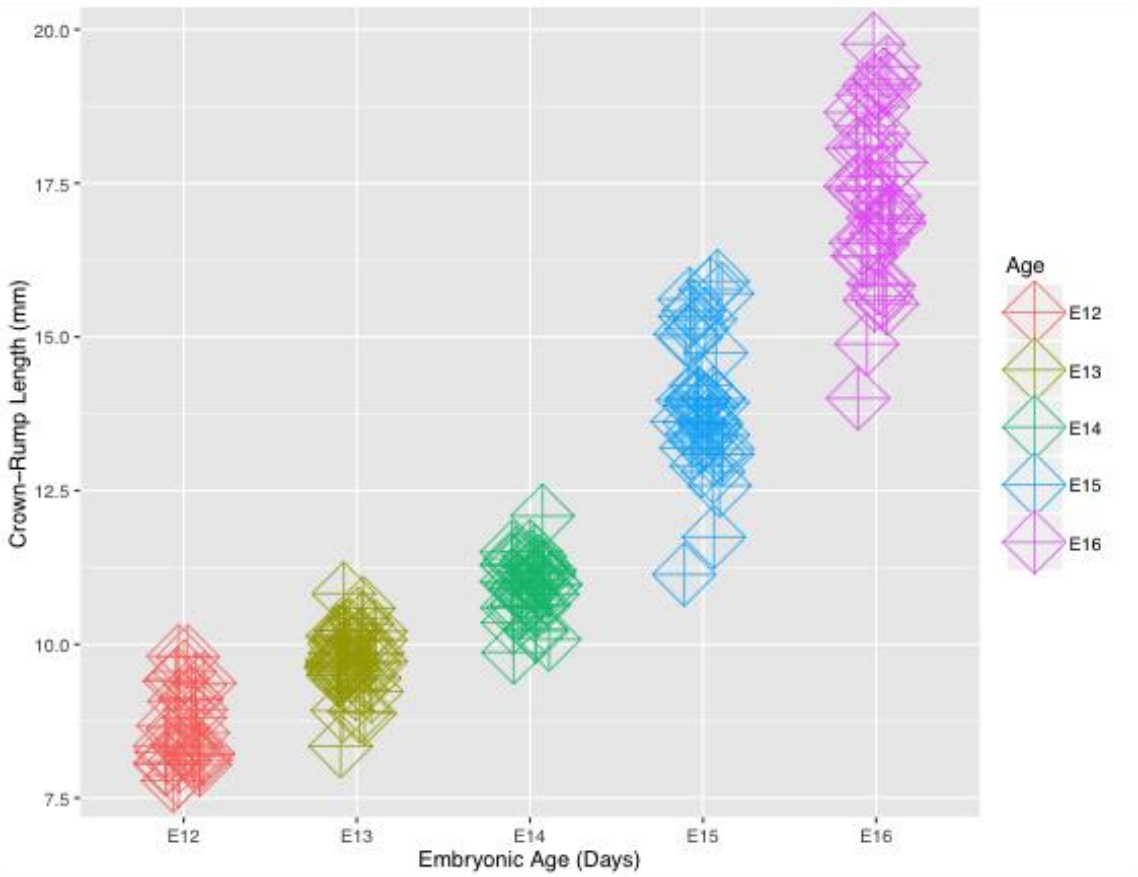


Figure 4 shows a scatter plot distribution of crown-rump lengths at each embryonic age showing the distribution of exact crown-rump measurements (mm) of specimen at each embryonic age. Embryonic age groups are plotted along the x-axis and differentiated by color. E15 and E16 specimens appear to have larger ranges of variation, while E12 - E14 specimens are more densely grouped. Specimen recorded at each age are as follows: E12 (n = 30), E13 (n = 42), E14 (n = 39), E15 (n =39), and E16 (n = 41).

Figure 5. Histogram and Density Curves for Crown-Rump Measurements

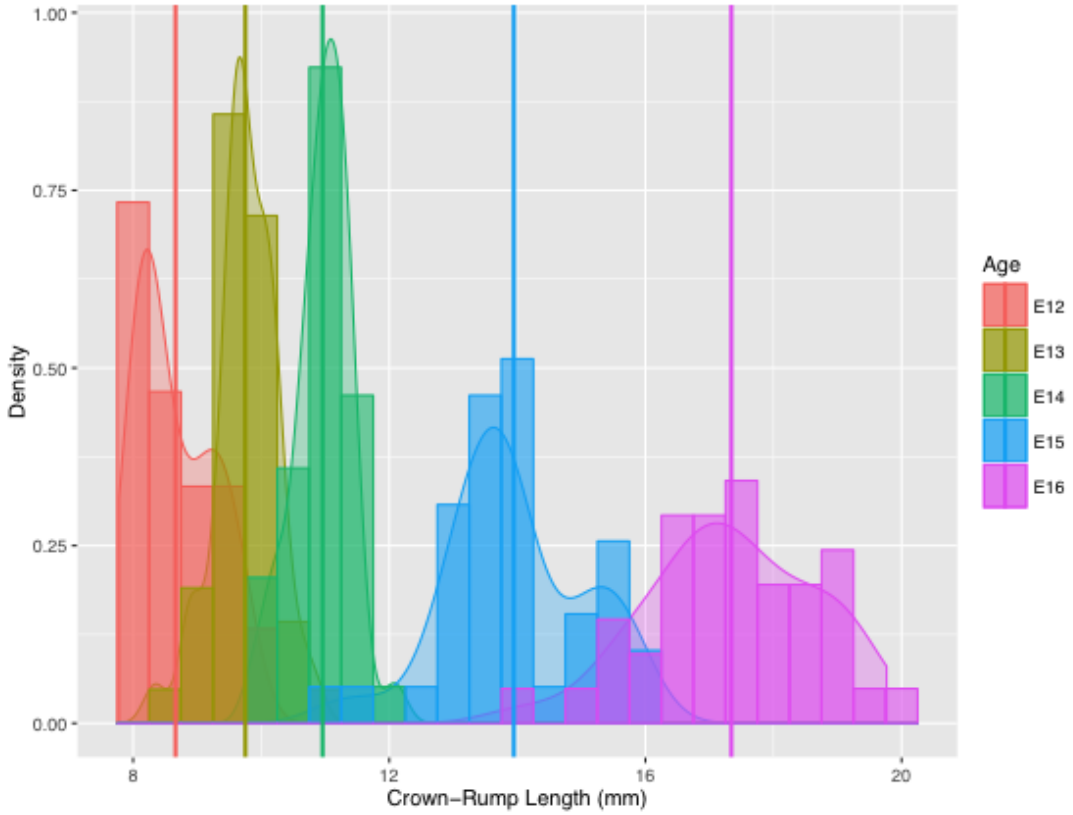


Figure 5 illustrates a histogram and density curves for crown-rump measures of individual mice E12 - E16. The density of crown-rump measurements is recorded along mm increments for each age. The vertical lines intersecting each color of the histogram represent the average crown-rump length for that particular age. The curved lines are probability density function curves for the distributions at each embryonic age. Sample size for each age are as follows: E12 (n = 30), E13 (n = 42), E14 (n = 39), E15 (n =39), and E16 (n = 41).

Digit Separation

Scores for the right hind limb of each embryo were categorized according to the classification system described previously (Table 2). Table 3 summarizes the distribution of digit scores across all developmental ages. Apart from E16, the limbs of mice at every embryonic day cross two classes of limb development. Specimen data clearly demonstrate overlap of the developmental phases of neighboring chronological ages.

Table 3. Number of Specimen Evaluated for Digit Score at Each Age

Table 3. Sample sizes for each age group are given in parentheses. These data are recorded from observation of the right hind limb.

	E12 *(n = 30)*	**E13** *(n = 42)*	**E14** *(n=39)*	**E15** *(n = 39)*	**E16** *(n = 41)*
0 	15	2	0	0	0
1 	15	23	1	0	0
2 	0	17	30	0	0
3 	0	0	8	17	0
4 	0	0	0	22	41

Whisker and Facial Follicle Presence

The results listed in Table 4 indicate that there is little intra-individual variation in these traits beyond E12. Embryos aged E13 and older show whisker follicle presence consistently. Because this is a binary trait, not much information can be collected beyond an estimated point of divergence between absence and presence; however, these data indicate that there is slight variation in the timing of whisker follicles appearance in prenatal development.

Table 4. Whisker Presence

Table 4 illustrates the number of specimen exhibiting whisker follicle presence (and absence) at each embryonic age studied.

AGE (n = number of specimen)	WHISKER FOLLICLE PRESENT	WHISKER FOLLICLE ABSENT
E12 (n = 41)	25	16
E13 (n = 48)	48	0
E14 (n = 44)	44	0
E15 (n = 44)	44	0
E16 (n = 59)	59	0

Table 5. Facial Follicle Presence

Table 5 illustrates the number of specimen exhibiting facial follicle presence (and absence) at each embryonic age studied.

AGE (n = total number of specimen)	WHISKER FOLLICLE PRESENT	WHISKER FOLLICLE ABSENT
E12 (n = 41)	14	27
E13 (n = 48)	39	9
E14 (n = 44)	44	0
E15 (n = 44)	44	0
E16 (n = 59)	59	0

In ages E14 - E15, all embryos were scored as possessing the two facial follicles. At embryonic ages E12 and E13, however, there is both presence and absence of this feature (see Table 5), meaning that the appearance of these features is likely completed in all developing mouse embryos by embryonic day 14 (E14). Like whisker development, information on facial follicle presence is temporally limited. If a binary trait is scored as being present, the embryo is only known to have survived the age of onset for the feature. That is, once the follicles are present within an embryo, not much other age information can be gathered from the trait.

Inter-trait Covariation

Crown-Rump Length and Digit Score

The relationship between crown-rump length and right hind limb digit score is shown graphically in Figure 6. These particular developmental traits appeared to be positively correlated. The variation observed steadily across age groups suggests that, based on the covariance of these two traits, developmental ages of mice collected on days E12 - E15 can potentially overlap with the developmental ages of mice up to 48 hours younger or older.

Figure 6. Scatter Plot of Crown-Rump Measurements and Digit Score Covariation

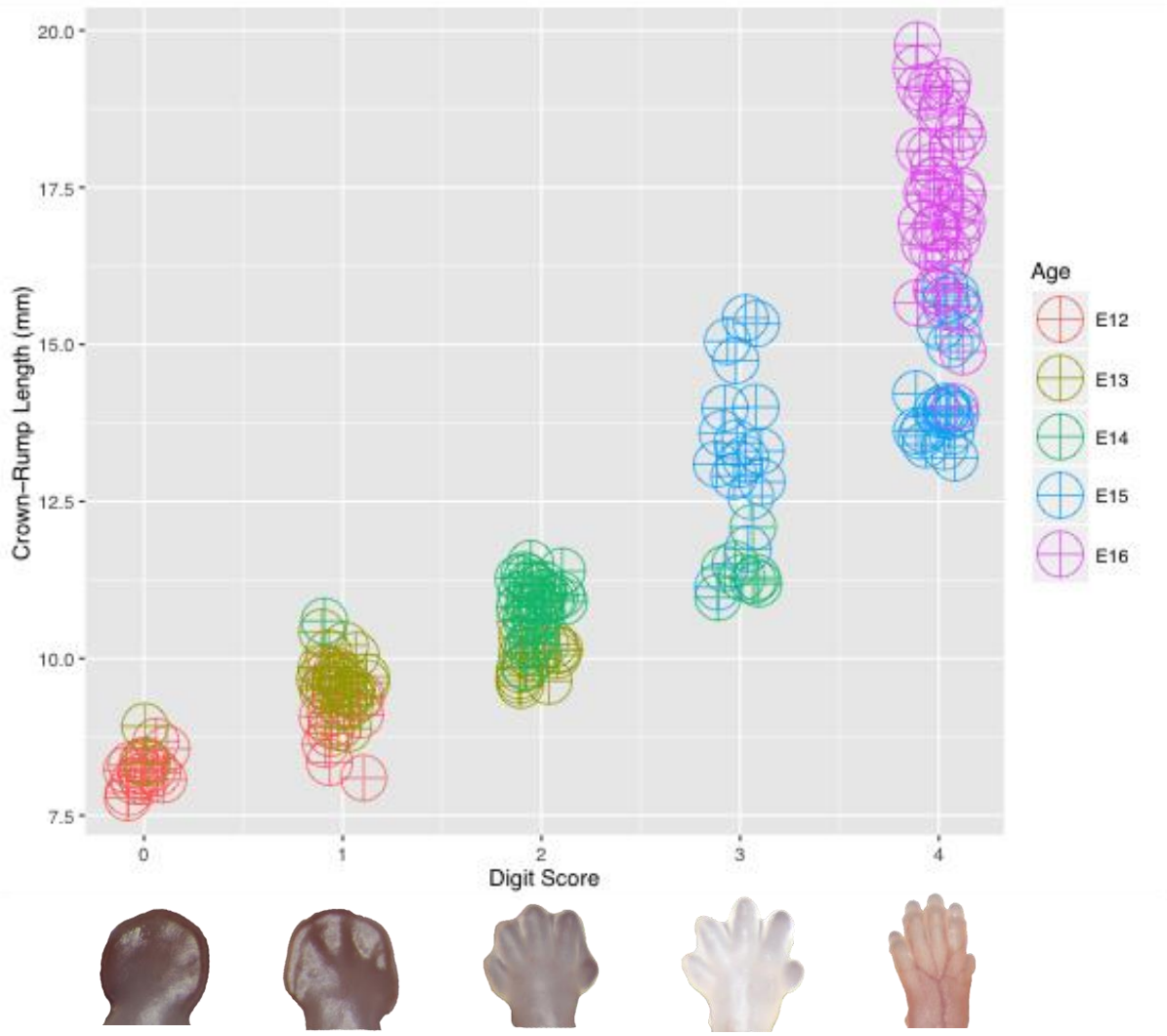


Figure 7. Scatter Plot of Crown-Rump Measurements and Digit Score Covariation. Associations between crown-rump length and right hind limb bud digit score expressed as a scatterplot. Each day of embryonic development is represented by a different color. Statistical analyses of these data were performed using the program R[15].

Chapter 4
Discussion

Using a sample of 298 mouse embryos, I compared the developmental features of embryos of known chronological age using several standard age-specific markers. I recorded crown-rump length measurements, whisker row and facial follicle presence/absence, and limb

bud digit score for each embryo. The results of this work show that developmental progress of mice collected on the same chronological day can overlap with the developmental ages of mice up to 48 hours younger or older. The results also indicate that the growth stages of different developmental markers do not align uniformly within individuals, as some systems may classify an embryo as belonging to a particular developmental stage while others may denote the individual as being more or less mature in terms of its developmental anatomy. Thus, the data provides evidence for both the overall variation between individual specimens as well as for the asynchrony occurring between the development trajectories of different anatomical systems within a single embryo. Although this research only explores data collected for embryonic ages E12 - E16, it is likely that these findings are consistent across other days of prenatal development. Future research may incorporate younger and/or older prenatal ages to evaluate patterns occurring across a wider span of prenatal development.

As expected, all individual traits observed exhibited some degree of variation within and between chronological age groups. These data provided different types of information about developmental age progression, but together contributed evidence demonstrating that variation exists for each feature used to characterize development within each day of prenatal development. Of the results, the correlation analysis between crown-rump length measurements and digit separation scores appear to be the most meaningful data. This illustrates the notion that although different anatomical systems of an embryo may be correlated in their growth patterns, they are still developing independently of one another and are hosts to widespread inter-trait variation.

In conclusion, one of the challenges facing developmental biologists using mouse models to characterize the gene and tissue interactions that coordinate craniofacial and other anatomic

regional development is the lack of a tight control on developmental timing in experimental contexts. Exact timing of important developmental events is difficult to estimate because of inter-individual variation in the rate of development and because these interactions take place during relatively brief timeframes. Accurate staging systems that recognize this naturally occurring variation are therefore crucial to the understanding of embryonic development and prenatal growth patterns. The data presented clearly illustrate the need for the creation of a more temporally precise and reliable system that is tied to various developmental systems. Ideally, this research will contribute to the establishment of new staging systems that encompass multiple measurable developmental traits and will provide more precise staging of mouse embryos than previously proposed methods. New techniques that capture the breadth of variation at a given chronological age allow researchers to address specific questions pertaining to individual variation in the timing and trajectory of development during this brief, yet critical, growth period.

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- Evolution of Human Parasites
- Biobehavioral Health
- Advances in Biological Anthropology Research
- Cultural Anthropology (Honors)
- Human Genetics (Honors)
- Mammalian Anatomy
- Biological Anthropology
- Elementary Statistics
- Biostatistics
- Zooarchaeology
- Human Sexuality and Evolutionary Biology
- The Skull
- Forensic Archaeology (Honors)

ANTHROPOLOGICAL FIELD EXPERIENCE

Smithsonian Natural Museum of Natural History, Washington, D.C.

Department of Anthropology — June 2015-August 2015

- Updated digital files for human skeletal database
- Cleaned, organized, and stored human burial remain collections
- Sorted possible human remains from extraneous burial material
- Created slides and skeletal identification cards for national Johnstown chancel burials press release

Department of Entomology — May 2014 – July 2014

- Managed department's computer database for reprint files
- Data entry using FileMaker Pro software
- Organized and maintained filing cabinets of print copies of reprint articles

The Pennsylvania State University's Craniofacial Morphometry Lab — January 2015 – present

- Work with embryonic mice models to examine developmental abnormalities, such as Craniosynostosis and Trisomy 21
- Assist grad student with mice model dissections and photographing images
- Plan to begin undergraduate thesis process in Spring 2015 semester

Carnegie Museum of Natural History, Pittsburgh, PA — August 2014

Department of Mammalogy

- Created isosurface images from CT scans of extant mammal species' bones for use in research projects
- Worked with AVIZO computer software

Consolidated Forensic Laboratory, Washington, D.C. — May 2014 – August 2014

District of Columbia's Department of Forensic Sciences, internship program

- Completed surveying research and managerial analysis of diversity in forensic science and STEM occupations
- Retrieved census data and created survey to record secondary data
- Currently managing survey and analyzing results for future publication under Dr. Max Houck, DFS Director

The Pennsylvania State University's Paleoanthropology Lab — January 2014 – present

- Small-scale animal dissections and necropsies for dermestid beetle decomposition
- Degrease and Peroxide animal skeletal remains for research use in various Penn State Anthropology labs
- General care of dermestid beetle colony and enclosure

The Pennsylvania State University's Bioarchaeology Lab — September 2013 – present

- Help graduate student collect data for doctoral dissertation
- Assist with data collection for development of new means of skeletal age estimation method for adult skeletons (Transition Analysis) through Dr. George Milner, Advisor
- Practice using various adult sex and age estimation methods on Native American skeletal remains

The Pennsylvania State University's Sexual Evolution & Anthropometry Lab September 2013 – Spring 2015

- Edited voice file recordings using PRAAT voice software
- Inputted data sets into Microsoft Excel software
- Assist with managing participant studies and campus surveys for lab research projects

GRANTS RECEIVED

Liberal Arts Enrichment Fund Recipient — Summer 2014, Summer 2015, Spring 2016
Erickson Discovery Grant Recipient — Summer 2015
Schreyer Honors College Internship Grant — Summer 2014, Summer 2015

PUBLICATIONS

Flaherty, Kevin, Vivianne Mazzocco, and J. T. Richtsmeier. 2015. "Patterns of Asynchrony between Developmental Age and Chronological Age in Utero." Baltimore, MD: abstract published Amer J Med Genet Part A.

EXTRACURRICULAR ACTIVITIES AND VOLUNTEER WORK

Intensive English Communication Program Tutor — Spring 2014-present
Global Connections: Conversation Partner Program — Fall 2012-Fall 2013
Children's Institute of Pittsburgh, Austin's Playroom — May-August 2011; June-August 2013
Special Olympics: Allegheny County — 2008-present

- Summer Games: 2008-present, *Executive Setup Committee*: 2009-2012
- Winter Games: 2009-2011

Habitat for Humanity, Bay-Waveland Area (Bay St. Louis, MS) — April 2011 and April 2012

SPECIAL SKILLS AND EXPERIENCE

Languages: Spanish-working level proficiency, Japanese-beginner level
Computer Skills: AVIZO, Microsoft Office, PRAAT Voice Software, Adobe Photoshop/Illustrator, Filemaker Pro, ArcGIS
Art: drawing, painting, ceramic sculpture, charcoal, fabric, metal, intaglio press/print making, screen-printing

EMPLOYMENT EXPERIENCE

Life's A Beach USA — June 2013-August 2013
Face painter/Special products

Whitehall Borough Recreation — June/July 2012, June/July 2013
Day camp counselor for kids ages 5-12