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The Tempo of Mammalian Embryogenesis: Variation in the Pace of Brain and Body Development

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Abstract

Why do some species develop rapidly, while others develop slowly? Mammals are highly variable in the pace of growth and development over every stage of ontogeny, and this basic variable – the pace of ontogeny – is strongly associated with a wide range of phenotypes in adults, including allometric patterns of brain and body size, as well as the pace of neurodevelopment. This analysis describes variation in the pace of embryonic development in eutherian mammals, drawing on a collected dataset of embryogenesis in fifteen species representing rodents, carnivores, ungulates, and primates. Mammals vary in the pace of every stage of embryogenesis, including stages of early zygote differentiation, blastulation and implantation, gastrulation, neurulation, somitogenesis, and later stages of basic limb, facial, and brain development. This comparative review focuses on the general variation of rapid vs. slow mammalian embryogenesis, with a focus on the pace of somite formation, brain vs. somatic development, and how embryonic pacing predicts later features of ontogeny.

Keywords

Embryo; Evolution; Brain; Evo devo; Development

Introduction: Evolving Development, Fast and Slow

A central way in which mammals differ from one another is in the pace of growth and development. Generally speaking, smaller mammals grow more quickly *in utero*, reach major developmental milestones more rapidly, are born after shorter gestation periods, reach sexual maturity earlier, and die from senescence at a younger age (i.e., r-strategy in life history theory [Pianka, 1970]). Larger mammals take longer to undergo all of these processes (i.e., K-strategy). This variation in the pace of growth and development scales allometrically across a diverse range of mammalian lineages. Mice develop faster than capybara, cats faster than lions, macaques faster than humans. How does evolution alter the pacing of mammalian ontogeny such that growth and development are coordinated,

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producing species that differ so widely in adult size? Which developmental mechanisms are evolvable enough to allow changes in the tempo of development within these diverse lineages of mammals?

Species differences in the pace of ontogeny are already evident during the earliest prenatal stages of ontogeny, and demonstrate a fundamental link between growth and development. Smaller mammals grow more rapidly in body size during prenatal development and reach major developmental milestones (e.g., gastrulation; neurulation; limb formation) more quickly [Butler and Juurlink, 1987]. Similarly, the tempo of brain development is accelerated in smaller mammals, requiring less time to produce brains of adult size and functional maturity [Workman et al., 2013; Halley, 2017; Sherwood and Gómez-Robles, 2017]. In fact, the relative size of brain areas has been described by allometric scaling rules that are thought to reflect basic variation in the tempo of a shared neurodevelopmental schedule (e.g., Workman et al. [2013]). How early do these differences in neurodevelopment emerge, and how do they relate to more global patterns of somatic development?

Many studies of heterochrony focus on how species differ in the development of specific body regions or cell types, and correct for more global differences in the pace of ontogeny in order to test for these focused differences. The aim of this review is instead to examine this background variation – the overall differences in the tempo of mammalian ontogeny that are usually “corrected for” using staging systems or other methods to establish developmental equivalence. A comparative dataset on the age, size, and morphology of mammalian embryos in fifteen species is used to characterize how growth and development vary during embryonic stages of ontogeny. This dataset is used to review overall patterns of growth and development across early, intermediate, and late stages of embryogenesis, and to revisit theories for how early developmental stages evolve. The present sample was selected based on the availability of standardized staging criteria applied to timed pregnancies; as such, it only reflects a fraction of eutherian mammalian species, and additional species are described when possible below.

Materials and Methods

Measurements of the age post-conception, crown-rump length (CRL), and morphology of embryos were assembled from published staging literature on embryonic development in fifteen species. The comparative dataset assembled by Butler and Juurlink [1987] was used to characterize the following 10 species, with primary sources listed in brackets: mouse [Theiler, 1972]; rat [Witschi, 1962]; hamster [Donkelaar et al., 1979]; guinea pig [Harman and Prickett, 1932]; rabbit [Marshall, 1893; Edwards, 1968; Hartman, 1974]; pig [Bryden et al., 1972]; sheep [Robinson, 1951; Bryden, 1969]; tree shrew [Kuhn and Schwaier, 1973]; marmoset [Phillips, 1976]; baboon [Hendrickx, 1971]; rhesus macaque [Hendrickx and Sawyer, 1975]. Data on human embryonic development was derived from O’Rahilly and Müller [2006]. Ages post-conception and associated stages for dog, cat, ox, and horse were assembled from Evans and Sack [1973].

Figure 1 shows the variation in species in the overall duration of the embryonic period. Each embryo was assigned a Carnegie Stage (CS) as a common system for comparison

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of developmental morphology, either via the studies above (e.g., Butler and Juurlink [1987]; O'Rahilly and Müller [2006]) or by applying the Carnegie Stage criteria to embryo descriptions (e.g., Evans and Sack [1973]). While the Carnegie staging system was originally developed to describe human embryos, it has subsequently been extended to a diverse number of mammalian species (e.g., Evans and Sack [1973]; Butler and Juurlink [1987]). Both the benefits and limits of this comparative approach are described at length below.

Figure 2a shows the time elapsed between CS9 (1–3 somites) and CS12 (21–29 somites). Figure 2b shows minimal and maximal estimates of the duration of somite-pair generation, calculated from somite number vs. the time elapsed from CS9 to CS12. Minimal somite-pair generation estimates assume that 29 somites are present at CS12 ([total duration]/29); maximal estimates assume that 21 somites are present at CS12 ([total duration/21]). Experimental values shown as stars in Figure 2b are taken from the following sources: chicken (90 min; Palmeirim et al. [1997]); mouse (120 min; Tam [1981]); human (240–300 min; Müller and O'Rahilly [1986]). Chicken is included as a point of comparison to mammalian species, given their central role in developmental biology and the availability of empirical measures for somitogenesis. Embryo diagrams in Figure 3 are adapted from photographs in Butler and Juurlink [1987].

The regression model in Figure 4c is an ordinary least-squares (OLS) regression model predicting stage per day from length (mm) per day, using the same data in Figure 4a and b. The regression model in Figure 5a is an OLS regression predicting brain development duration from the age difference between CS9 and CS12. Brain development duration was calculated from models of neurodevelopmental event timing in Workman et al. [2013], specifically the differences between estimates of the earliest model event (onset of trigeminal ganglion cell generation) and latest event (end of myelination in the middle cerebellar peduncle). The regression model in Figure 5b is an OLS regression predicting adult brain size from the age difference between CS9 and CS12. Adult brain sizes are taken from an earlier review of the literature [Halley, 2016]. Lateral brain diagrams in Figure 5c are redrawn from photographs from the Comparative Mammalian Brain Collection, University of Wisconsin.

Results

General Patterns in the Pace of Mammalian Embryogenesis

The duration of the embryonic period ranges from 16 days in mouse to 56 days in human (Fig. 1a) in a sample of fifteen eutherian mammals for which embryogenesis has been well described. This sample includes carnivores, ungulates, rodents, tree shrew, and primates. Broadly speaking, smaller species within this sample exhibit shorter embryonic periods, while larger species have extended periods of embryogenesis. Figure 1b shows the same embryonic period data from Figure 1a, but reoriented around Carnegie Stage 12 (CS12), a stage of advanced somite and neural development. This alternative orientation shows that mammals differ in the duration of both early (left, CS1>12) as well as later stages of embryogenesis (right, CS12>23).

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What developmental events occur over these stages, and how do they vary among mammals? The following sections examine how mammals vary in the pace of early (CS1>9), intermediate (CS9>12), and later stages (CS12>23) of embryonic development. Though these periods are relatively arbitrary, they were chosen to distinguish the intermediate period of somitogenesis from preceding and subsequent stages, and their comparison can highlight important features that vary between species.

Variation in the Pace of Early Mammalian Embryogenesis (CS1>CS9)

In mammals, differences in the total duration of early embryonic development are thought to occur in three distinct ways: delayed fertilization (i.e., sperm storage), delayed implantation (i.e., diapause), and delayed development [Renfree and Shaw, 2000]. Delayed fertilization (i.e., sperm storage) is common in reptiles and birds, but seems to play a relatively minor role in how mammals vary in the duration of gestation [Birkhead and Møller, 1993] with the exception of certain bat species (e.g., Gopalakrishna and Madhavan [1971]; Myers [1977]; see Sandell [1990] and Birkhead and Møller [1993] for adaptive theories of bat sperm storage). Accordingly, this section focuses on variation caused by delays in implantation and decelerations in mammalian development. Note that while both processes alter the duration of the early embryonic period, only deceleration alters the pace of developmental events.

Following fertilization (CS1), the zygote undergoes a series of cell divisions that results in a collection of ~16 cells called the morula (CS2, 3). Like other animal lineages, in mammals these cell divisions involve relatively little cellular growth, such that the initial cellular mass of the zygote is divided into daughter cells without a substantial increase in overall size [Tadros and Lipshitz, 2009]. The morula then begins an asymmetrical process of cell division (blastulation; CS4, 5) that concentrates cells along one pole – the inner cell mass – forming a blastula. Further differentiation of the inner cell mass of the blastula produces three distinct cell layers that will constitute the adult organism (gastrulation; CS6, CS7).

One of the central forms of variation in the duration of early mammalian embryogenesis is delayed implantation, or diapause. This occurs when an embryo undergoes a pause in cell division, cell growth, or both at the blastocyst stage of development, prior to implantation in the uterine wall (CS5) [Renfree and Shaw, 2000]. Diapause has been observed in over 130 species, including at least ten distinct orders of mammals – primarily carnivores, rodents, and marsupials, but also certain species of bats, shrews, armadillos, and artiodactyls (reviewed in Fenelon et al. [2014]). Diapause can last anywhere from several days (e.g., mice, rats) to 10–11 months in some badgers or wallabies [Yamaguchi et al., 2006; Renfree and Shaw, 2014]. Depending on the species, the control of diapause involves a combination of endocrine signals from the mother (facultative diapause) and the external environment (obligate diapause) [Lopes et al., 2004], both of which can allow species to control the timing of birth independently of when mating occurs [Renfree and Fenelon, 2017]. Interestingly, diapause has been artificially induced by the transfer of sheep embryos (which do not normally undergo diapause) into mice with diapause conditions [Ptak et al., 2012]. This capacity to induce diapause from the local environment suggests that the control of diapause onset relies on local signaling mechanisms, rather than the genotype of the developing embryo. This may help to explain its presence in a diverse range of eutherian

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and metatherian species. As noted above, implantation delay is an important contributor to differences in the *duration* of early embryonic stages, but does not necessarily alter the *pace* of morphological development before or after implantation occurs.

In the current dataset, the elapsed time from fertilization (CS1) to implantation and the onset of gastrulation (CS6) is widely variable: 5–7 days in mouse, rat, guinea pig, and rabbit; 8–11 days in pig and sheep; and 10–16 days in baboon, macaque, and human. Following implantation, the process of gastrulation generates three differentiated layers of cell types – ectoderm, mesoderm, and endoderm (CS6, CS7). The derivatives of two layers are discussed at length below. First, portions of the mesoderm will produce somites along the rostrocaudal axis of the embryo, transient embryonic structures that have directly informed our understanding of how embryonic segmentation evolves. Second, portions of the ectoderm form two cell populations that will compose the adult nervous system: the neural tube and neural crest. The neural tube is formed from the initial fusion of the neural folds (CS10) and the subsequent closure of the rostral (CS11) and caudal neuropores (CS12). The completion of this process of neurulation distinguishes the ectoderm derivatives that will compose the central nervous system, while neural crest cells contribute to the peripheral nervous system (among other derivatives).

The following sections examine changes in the overall pace of embryogenesis and downstream developmental events. These changes can be described either as acceleration or deceleration, depending on the reference point; they can include either specific body parts (e.g., “faster forelimb development”) or coordinated changes to the pace of development of the overall embryo (e.g., “fast vs. slow species”). All are cases of heterochrony, but with different implications for both evolution and developmental mechanisms. Below, we examine examples of tissue-specific heterochrony (e.g., limb development in marsupials), but focus especially on the more global patterns of variation – how mammals vary in the overall pace of embryogenesis, producing “fast” and “slow” developmental phenotypes in general.

Variation in the Pace of Mammalian Somite Formation (CS9>CS12)

One of the best-studied stages of vertebrate embryogenesis is the period of early somite formation (CS9>CS12), a period which overlaps with the appearance of pharyngeal arches and the upper limb buds. Somites are transient embryonic structures derived from the mesoderm, generated in sequence along the rostrocaudal axis of the embryo. The cells that constitute individual somites will eventually develop into vertebrae, ribs, cartilage, muscle, and skin along this axis. Somitogenesis is especially important in comparative embryology – it is a developmental process that is shared across vertebrates, and which produces discrete, sequential, and quantifiable phenotypes (somite pairs). While experimental studies of somitogenesis are limited to model species (see below), descriptive studies of somite development and derivatives have been much broader. One window into the evolutionary variation of this process comes from comparative studies of a somitic derivative that is more easily studied in adult organisms – the vertebrae. The number of vertebrae in adults corresponds to the number of somites in the embryo of a given species of vertebrate, and this number is remarkably variable, ranging from <10 in frogs [Young, 1962] to 300+ in certain

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species of snakes [Gomez et al., 2008]. See Richardson et al. [1998] for a review of this variation.

Within the Carnegie System, 1–3 somites appear at CS9, 4–12 somites appear at CS10, 13–20 somites appear at CS11, and 21–29 somites appear at CS12. While the duration of somitogenesis has only been measured directly in a few species, we can estimate this variation by comparing staging studies in embryos of known age (Fig. 2). Figure 2a shows that most rodents produce ~30 somite pairs in 1–2 days, while ungulates take 3–5 days, carnivores take 4–6 days, and primates take 5–7 days to produce the same number. This variation between species in the pace of somitogenesis mirrors the variation in overall embryonic development (Fig. 1a).

Figure 2b shows estimated ranges for the time required to produce a single pair of somites in each species, based on elapsed time from CS9 to CS12 from staging studies. These approximations show minimum and maximum values, based on calculations that assume either 21 or 29 somites at CS12, and do not incorporate differences in the duration of early- vs. late-somite generation. Extreme values in some species (e.g., dog, macaque) may be artifacts of data availability from the staging studies on which this analysis is based, rather than genuine variation.

With those qualifications in mind, Figure 2b shows the variation in estimated time required to generate a single pair of somites – ranging from ~75 minutes in chicken and rodent species to 300+ minutes in primates and carnivores. Empirically derived data in chicken [Palmeirim et al., 1997], mouse [Tam, 1981], and human [Müller and O'Rahilly, 1986] are close to the estimated ranges predicted from staging data alone (see stars for each species in Fig. 2b). Even these rough estimates show that mammals vary widely in the pace of somitogenesis, a temporal phenotype that matches overall variation in embryonic pacing (Fig. 1a).

What mechanisms are involved in producing this variation in the tempo of somitogenesis, and how do they vary between species? A major hint to this evolutionary variation has come from developmental manipulations of somite formation. Even when embryos are experimentally reduced in size prior to somitogenesis, a normal number of somites are produced [Waddington and Deuchar, 1953; Cooke, 1975]. To explain this constancy, Cooke and Zeeman [1976] proposed the "clock-wavefront" model of somitogenesis. Briefly, their model proposed that presomitic cell populations include a molecular "clock" – an oscillating sequence of intracellular gene expression in presomitic cell populations which is interrupted by the arrival of a rostrocaudal "wavefront" of gene expression. As the wavefront signal moves along the embryo from rostral to caudal, it generates discrete cellular units (somites) according to the state of the "clock" oscillation of cells it passes. Essentially, the model describes a flexible mechanism for cellular differentiation that can account for the remarkable constancy among individuals within a species. Importantly for evolutionary differences, changes to the model's basic components (e.g., clock oscillation, wavefront speed, rostrocaudal length) may be responsible for how species differ in the number and size of somites, as well as their pace of emergence.

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Recent empirical studies have supported the clock-wavefront model, and point to a particular mechanism – the clock oscillation – as a major source for evolutionary variations in somitogenesis. Gomez et al. [2008] found experimental evidence for the clock-wavefront model by measuring gene expression during somitogenesis in a comparative sample of fish, reptiles, birds, and mammals. First, they confirmed a major prediction of the clock-wavefront model: the rostrocaudal distance covered by the wavefront during a single clock oscillation corresponds to the size of the somite produced. Next, they focused on the exceptional number of somites in corn snake (~315) relative to zebrafish (31), chicken (55), and mouse (65). Taking into account differences in the number of somites and their rate of formation, they found that the segmentation clock was ~4 times faster in snakes than in other species.

If changes to the clock are responsible not only for extreme cases (e.g., snake) but also more general variation in somitogenesis, as we see in mammals, what mechanisms might be responsible? Two recent in vitro studies have used pluripotent stem cells to examine species differences in the pace of somitogenesis, pointing to changes in the pace of protein metabolism. Matsuda et al. [2020] used in vitro models to examine the basis of clock duration in mice (2–3 h) vs. humans (4–6 h). Surprisingly, the speed of the clock was not associated with uniquely human variations in gene regulatory networks (GNRs) involved, but instead a slower rate of biochemical reaction speed involved in protein degradation. Similarly, Rayon et al. [2020] used in vitro methods to show that while mouse and human GNRs were similar, a human-specific delay in the generation of motoneurons was associated with a longer half-life of proteins. While in vitro studies can only model the process of embryogenesis, these studies suggest that the pace of development is linked to variation in protein metabolism, even as it remains unclear how and when these metabolic differences between species first emerge at a genetic level [Matsuda et al., 2020].

Marsupials present a unique case of variation in the timing and pace of somitogenesis. In general, marsupial brains and bodies both grow and develop more slowly than eutherians, a phenomenon that has been well described [e.g., Renfree et al., 1982; Halley, 2017] even as it remains poorly understood. Part of this overall deceleration in marsupial growth and development is a slower pace of somitogenesis, as demonstrated in *Monodelphis domestica* [Keyte and Smith, 2012]. However, *Monodelphis* also produces its first cervical somites *earlier* during development than expected relative to eutherians [Keyte and Smith, 2012], even as caudal somitogenesis is delayed as part of generally slow schedule. This basic pattern of rostral acceleration vs. caudal deceleration is reflected in marsupial limb formation. The acceleration in cervical somitogenesis is coupled with an earlier development of forelimb buds, which emerge from adjacent portions of the rostral embryo [Keyte and Smith, 2010]. Meanwhile, the overall deceleration of somitogenesis (especially in caudal/tail somites) is reflected in a delay of hindlimb development [Sears, 2009]. Instead of limb heterochrony changing in concert (e.g., an earlier onset of both fore- and hindlimbs), each limb develops in concert with adjacent tissue types according to shared signaling (i.e., a more global mechanism that is setting the pace of development across the rostrocaudal axis of the embryo). The acceleration of forelimb development is an adaptation that allows marsupial neonates to migrate to the pouch or nipples, despite their highly altricial state at birth [Keyte and Smith, 2010].

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The Question of a "Phylotypic" Stage of Vertebrate Embryogenesis (CS9>CS12)

This intermediate stage of embryonic development overlaps with a period of embryonic development, the so-called "phylotypic stage," that has been argued to be shared among diverse invertebrate and vertebrate species that compose the kingdom Metazoa [Slack et al., 1993]. While the concept of a phylotypic stage of embryogenesis has its origins in Haeckel's disproven theories of recapitulation, the idea of a conserved stage of animal embryogenesis was revisited with the introduction of new methods for studying gene expression in diverse species.

Briefly, many of the genes expressed during the "phylotypic stage" of embryogenesis (e.g., HOX genes) are evolutionarily conserved in both their sequence and spatial expression [Gerhart and Kirschner, 1997], suggesting that morphological similarities observed by early studies may in fact reflect genetic programs for body patterning that have been conserved in evolution (e.g., Slack et al. [1993]; Irie and Sehara-Fujisawa [2007]; Kalinka et al. [2010]; Irie and Kuratani [2011]). The modern theory that intermediate stages of embryonic development are more conserved across species – "phylotypic" – while earlier and later stages are more variable, has been referred to as the "hourglass model" of comparative embryology (e.g., Gerhart and Kirschner [1997]). This renewed theory that a conserved "phylotypic" stage exists has been challenged on the basis of comparative analysis of embryo collections (e.g., Richardson et al. [1997]; Bininda-Emonds et al. [2003]) but has also been supported recently by evidence that gene expression is conserved during this stage of development (e.g., Kalinka et al. [2010]; Irie and Kuratani [2011]). Whether a conserved stage of embryonic development exists is subject to ongoing debate in comparative embryology (c.f. Richards [2009]; Richardson [2012]).

Variation in the Pace of Late Mammalian Embryogenesis (CS12>CS23)

Just as mammals differ in the duration of early and intermediate stages of embryonic development, later stages of embryonic development (CS12–23) are highly variable in their duration. Within the Carnegie system, embryos of this period are assigned stages according to externally visible features across a range of body regions, including the limbs, eyes, ears, nasal cavities, cranium, and trunk (e.g., O'Rahilly and Müller [1987]).

Limb development helps to define many of the later stages of the Carnegie System. Forelimb buds appear at CS12, form a "hand plate" at CS15, "finger rays" at CS17, a distinct elbow at CS18. After this, most forelimb criteria are fairly subjective, and difficult to apply across species (e.g., "fingers longer"; "hands approach each other"). A similar but delayed progression of hindlimb events help to define each stage: hindlimb buds are present at CS13; the foot plate at CS16; the toe rays at CS18. In eye development, the optic vesicle (CS11) develops into a lens disc (CS13), lens pit and optic cup (CS14), and lens vesicle (CS15); the final stage is the appearance of retinal pigment from outside the embryo (CS16). Ear development involves the development of the otic vesicle (CS13) into distinct appendages (CS14). Nasal formation includes the emergence of the nasal pit (CS15) and nasofrontal grove (CS17).

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Species differ widely in both the duration and tempo of these later embryonic events. Generally speaking, smaller species (e.g., rodents) develop faster, and larger species (e.g., primates) develop more slowly. Figure 3a compares the duration of later embryonic development in five species of mammals (mouse, guinea pig, pig, rhesus monkey, and human), a period that ranges from ~5 to 25 days. Every stage of this period is variable across species – the grey lines in Figure 3a connect equivalent stages, showing how the entire process of development is compressed or stretched across species.

In practice, the limits of the Carnegie Stages (or any other comparative system) to align different species' embryos become especially apparent after CS17/18. Figure 3b shows how lineage-specific characters emerge during this period (e.g., whiskers in rodents), comparing the lateral perspective of five species at five stages of later development. Species variations are increasingly apparent at CS17>23, and complicate the effort to establish "equivalence" over these stages. Further, the morphological criteria used to define Carnegie Stages after CS17/18 become more subjective (e.g., CS21: "hands and feet approach each other"). Even within humans, the limit of meaningful age equivalence (i.e., stages) is marked by the categorical boundary between "embryonic" and "fetal" stages of development (~56–60 days post-conception) in embryology. These limits to embryonic staging (or any other system of comparative ontogeny) are examined below.

One useful aspect of the assignment of stages in later embryogenesis is to demonstrate how evolutionary changes to morphogenesis are linked to changes in the pace of growth. Species that grow more slowly, such as humans, also take more time to progress from one embryonic stage to the next. Figure 4a shows how mammals differ in the rate of growth (embryo length in mm) relative to chronological age (data are aligned according to the age of CS12 rather than fertilization to ease comparison). As we have seen previously, smaller mammals (e.g., rodents) grow more rapidly during embryonic development, while larger species (e.g., primates) grow more slowly. This variation in growth rate (Fig. 4a) matches species variation in the pace of morphological development, shown in Fig. 4b. Essentially, smaller species (e.g., rodents) grow and develop more rapidly, while larger species (e.g., primates) grow and develop more slowly (Fig. 4c).

Discussion

The Pace of Mammalian Embryogenesis Relative to Later Ontogeny

While individual cases of heterochrony in particular body regions have been described above, when we broadly compare the pace of embryogenesis across mammals (Fig. 1), the most basic form of variation involves a coordinated change to the pace of development that affects the overall embryo and its diverse tissue types in concert. These differences in the pacing of mammalian embryonic growth and development – "fast" and "slow" species – are linked with phenotypic differences in the pace of later fetal, postnatal, and adult stages of ontogeny. Included in this suite of associated phenotypes are the classic allometric patterns of mammalian variation, including brain/body scaling and brain area scaling.

One way of demonstrating this coordination is to compare the developmental pacing of distinct tissue types across species, such as somites and the brain. Even though somites do

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not contribute to the brain, the period of somitogenesis predicts both the duration of brain development (Fig 5a) and adult brain size (Fig 5b) across a range of brain sizes (Fig 5c).

Why? Essentially, a common developmental program is setting the pace of diverse embryological events, which include neurological and somitic precursors, but also other tissue types. This overall pacing in embryonic development is associated with a larger suite of phenotypes that track onto a wide range of downstream phenotypes: gestation length, neonatal brain and body size, adult brain composition, age of sexual maturity, life span, etc.

The Pace of Mammalian Embryogenesis in Phylogenetic Context

This analysis has focused on eutherian species whose embryonic stages have been described using timed conceptions and standardized staging criteria. As noted earlier, staging studies require that embryos are collected from timed pregnancies, which has limited the number of mammalian lineages for which detailed data on age, stage, and size can be collected. For example, the current analysis includes staging data for 7 of the 18 eutherian Orders (*Rodentia*, *Scandentia*, *Lagomorpha*, *Carnivora*, *Perissodactyla*, *Cetartiodactyla*, *Primates*), as timed-pregnancy data is scarce or non-existent for other lineages. However, it is worth noting more limited datasets in species not described above – either individual data points, or species whose embryos have been categorized using different staging systems.

In primates, several reports suggest that embryonic development is extended in both the lesser galago (61–61d [Butler and Juurlink, 1987]), and marmoset (90+d [Phillips, 1976]) due to delays in early embryonic development. Recent developmental studies in microbats (e.g., Cretekos et al. [2005]; Nolte et al. [2009]) demonstrate a much slower pace of growth and development relative to similarly sized mammals (e.g., rodents), including in brain development [Martínez-Cerdeño et al., 2018]. Finally, marsupial prenatal development shows a number of unique characters relative to eutherians. In addition to the heterochronies in somitogenesis and limb development described above, marsupials exhibit a general deceleration in growth that has been well characterized in both the brain and body [Renfree et al., 1982; Workman et al., 2013; Halley, 2017].

Staging and Age Equivalence in Comparative Ontogeny

A central problem in comparative ontogeny is the question of how to assign “equivalent” stages of development in diverse species (e.g., Workman et al. [2013]). In comparative embryology, a diverse number of staging systems have been proposed for particular species and lineages, with variable criteria used to define stages in any given system. The Carnegie Staging system used here was originally developed to standardize human embryos that shared morphology but differed in age post-conception [O’Rahilly and Müller, 1987]. It was subsequently extended to other mammals for a parallel purpose, one used in this analysis: to produce a shared system of categorization based on phenotypic similarities, independent of the large differences in post-conception age between species (e.g., Butler and Juurlink [1987]).

Both the usefulness and the limitations of the Carnegie System derive from its “low resolution” in relation to embryological events [Bininda-Emonds et al., 2002]. Using a common system of classification allows us to see how mammals differ in both the overall

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pace of development (Fig. 4a) and growth (Fig. 4b), as well as how these variables are correlated (Fig. 4c). However, this approach also obscures differences in how species develop individual organs and tissue types [Richardson, 1995]. Especially during later stages of embryogenesis, staging systems fail to capture how species differ in the progression of different tissue types with distinct embryonic origins (e.g., pharynx, somites, eye, ear, cranium, forelimb, hindlimb, etc.). These limits become especially apparent during later stages, as species-unique phenotypes emerge (e.g., vibrissae in rodents) and stage criteria become more subjective (e.g., CS23, as defined in humans: “limbs longer and more developed”). Alternative approaches to comparative embryology have been developed to capture this variation, such as the use of sequential event pairing [Smith, 2001, 2002] – an especially useful approach when the age post-conception of a given embryo is unavailable.

One way to avoid the inherent “conservatism” of staging systems – i.e., their tendency to miss species-unique heterochrony in particular tissues – is to forgo embryonic staging altogether. In species for which the age post-conception of developmental events is known, tissue-type event timing can be compared both within and across species to test for heterochrony. This method has been successfully used by Finlay and colleagues in their comparative analyses of neurodevelopment (e.g., Clancy et al. [2001]; Workman et al. [2013]). While this method depends on the existence of aged-event data or model extrapolations, it has never been applied more broadly to embryonic structures outside of the brain, and offers a way beyond the limits of staging in order to test for heterochrony in comparative datasets.

Zooming Out: Evolutionary Strategies and the Question of Mechanism

How does evolution alter development to produce species with smaller or larger bodies and brains? The existence of size variation within each mammalian lineage – small and large rodents, carnivores, primates, etc. – suggests that size is particularly “evolvable” as a phenotype. While mechanisms can only be determined from experimental studies in particular species, a comparative approach to the literature of staged embryology can offer insights into when during ontogeny differences in developmental pacing emerge, and which tissues they affect.

This review shows that the differences in developmental pacing that distinguish species later in ontogeny (e.g., adult body or brain size) are already present at the earliest stages of embryonic development. Larger species with larger brains have longer periods of embryogenesis (Fig. 1a, b). Larger species take more time to produce their first 30 somites (Fig. 2a), and each somite pair is produced more slowly (Fig. 2b). Even after somite formation is complete, larger species continue to grow more slowly (Fig. 4a) and develop more slowly (Fig. 3a, 4b). These early differences in embryonic pacing are associated with a wide suite of phenotypes that distinguish species from one another later on in ontogeny: the pace of fetal and postnatal growth, gestation duration, and the size and allometric composition of the brain, among others. In evolutionary terms, this axis of variation is associated with the traits that define r- and K-selection strategies, including litter size, parental investment, relative altriciality at birth, and the timing of life history events (e.g., sexual maturation). In essence, mammalian body size and its long list of associated

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phenotypes are already evident at the earliest stages of embryogenesis, and we do not yet understand the developmental mechanisms responsible.

In studies of brain evolution, it has long been known that species differ in the composition of brain areas, and that these allometric differences are associated with differences in the duration of neurodevelopment (e.g., Finlay and Darlington [1995; Workman et al. [2013]). One motivation of the current review has been to compare these differences in the pace of neurodevelopment with more global changes to overall embryonic morphogenesis. Briefly, it appears that evolutionary changes to the pace of brain development are downstream effects of more global changes that affect every part of the body. This is indicated by the observation that the pace of events outside the brain, like somite formation, are predictive of the duration of neurodevelopment (Fig. 5a) and subsequent adult brain size (Fig. 5b). This shows that the pace of brain development is only one aspect of a larger variation in the tempo of global embryonic development, one which affects the timing of diverse tissue types, and the mechanisms for which have yet to be elucidated.

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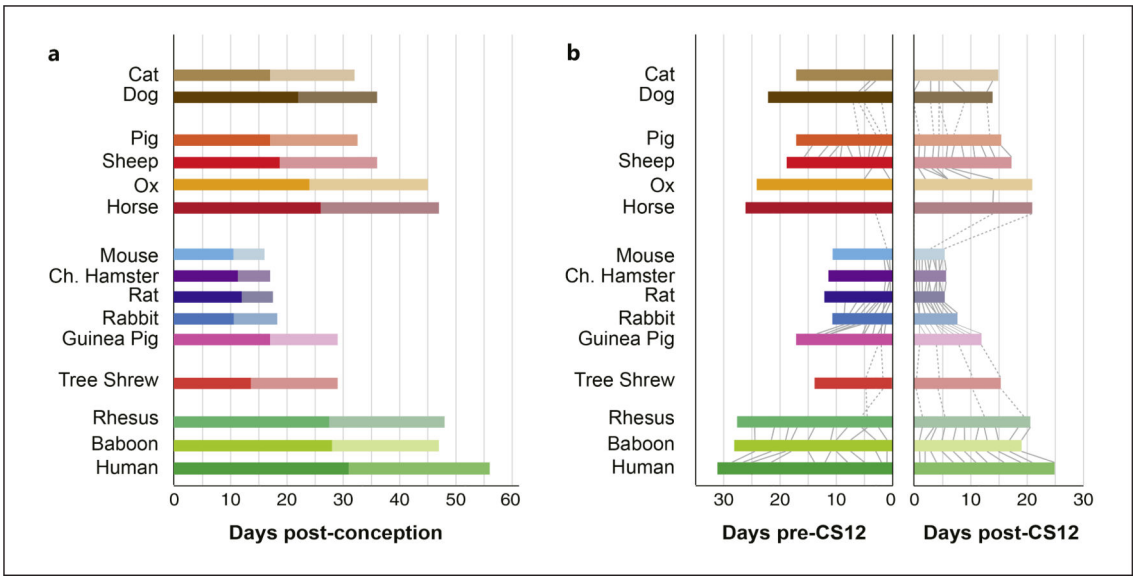

Fig. 1.

Variation in the total embryonic period in mammals. **a** The duration of the embryonic period of development (Carnegie Stages [CS] 1–23) in fifteen mammalian species. Embryonic period duration is measured in days post-conception, as estimated from staging studies (see below). For each species, the darkly-shaded portion of each bar indicates CS1–12, while the lightly shaded portion of each bar indicates CS12–23. Mammals vary widely in the duration of the embryonic period. The species shown here range from 16 days (mouse) to 56 days (human), and exceptionally long periods have been reported in other species (not shown; the lesser galago, 61–61d [Butler and Juurlink, 1987], marmoset, 90+d [Phillips, 1976], and several bat species, 70+d [Cretekos et al., 2005; Nolte et al., 2009]). **b** The same staging data represented with CS12 as the *y*-axis origin. Lines between each species' bar connect equivalent stages, where available. Data sources, as well as a description of Carnegie Staging as a comparative tool, are described in the Methods section.

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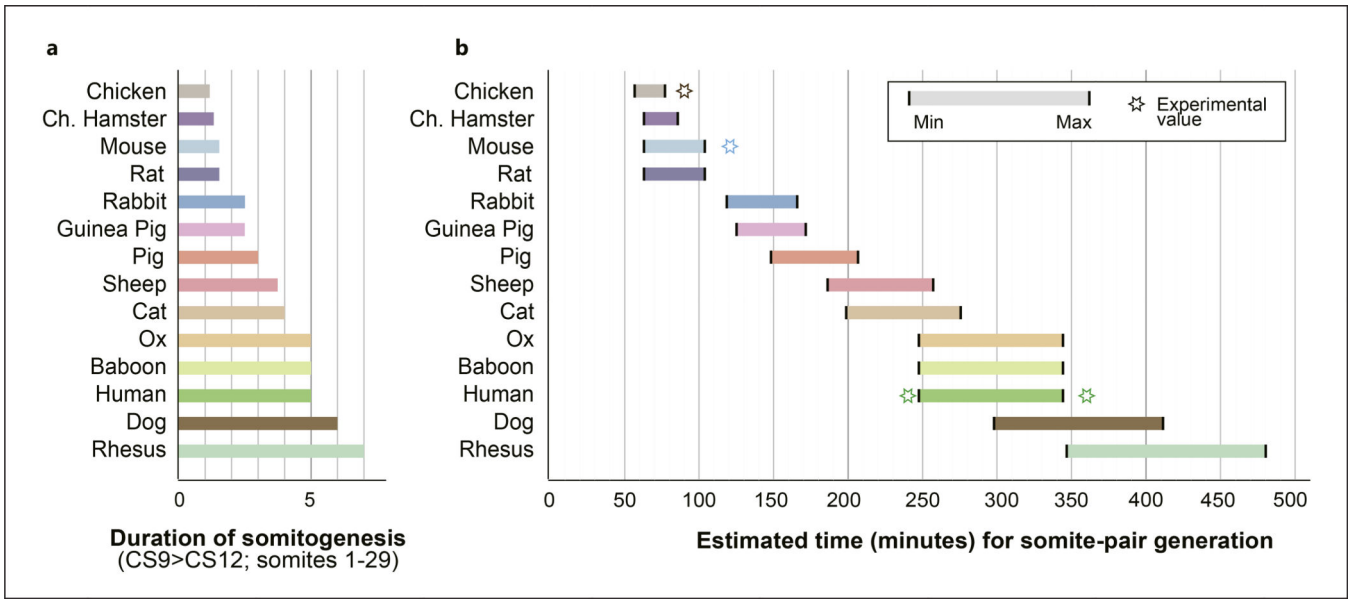

Fig. 2.

Variation in the duration of intermediate stages of mammalian embryogenesis (CS9>12) and estimated duration of somite pair generation. **a** The duration of early somitogenesis (CS9>12) in 14 species, based on staging studies. This period encompasses the stage of 1–3 somites (CS9) through 20–29 somites (CS12). Species are aligned using the age post-conception of CS9 as the origin, and are sorted according to total duration. The duration of this period is highly variable, taking anywhere from 1–2 days (e.g., chicken, rodents) to 5–8 days (primates). **b** Estimates of the time in minutes required for each species to generate a single pair of somites, based on the duration of CS9>CS12. Minimum and maximum duration estimates are calculated by assuming CS12 represents 29 vs. 21 somites, respectively (see Methods). Stars indicate empirical values on the duration of somitogenic pair generation in chicken [Palmeirim et al., 1997], mouse [Tam, 1981], and human [Müller and O'Rahilly, 1986].

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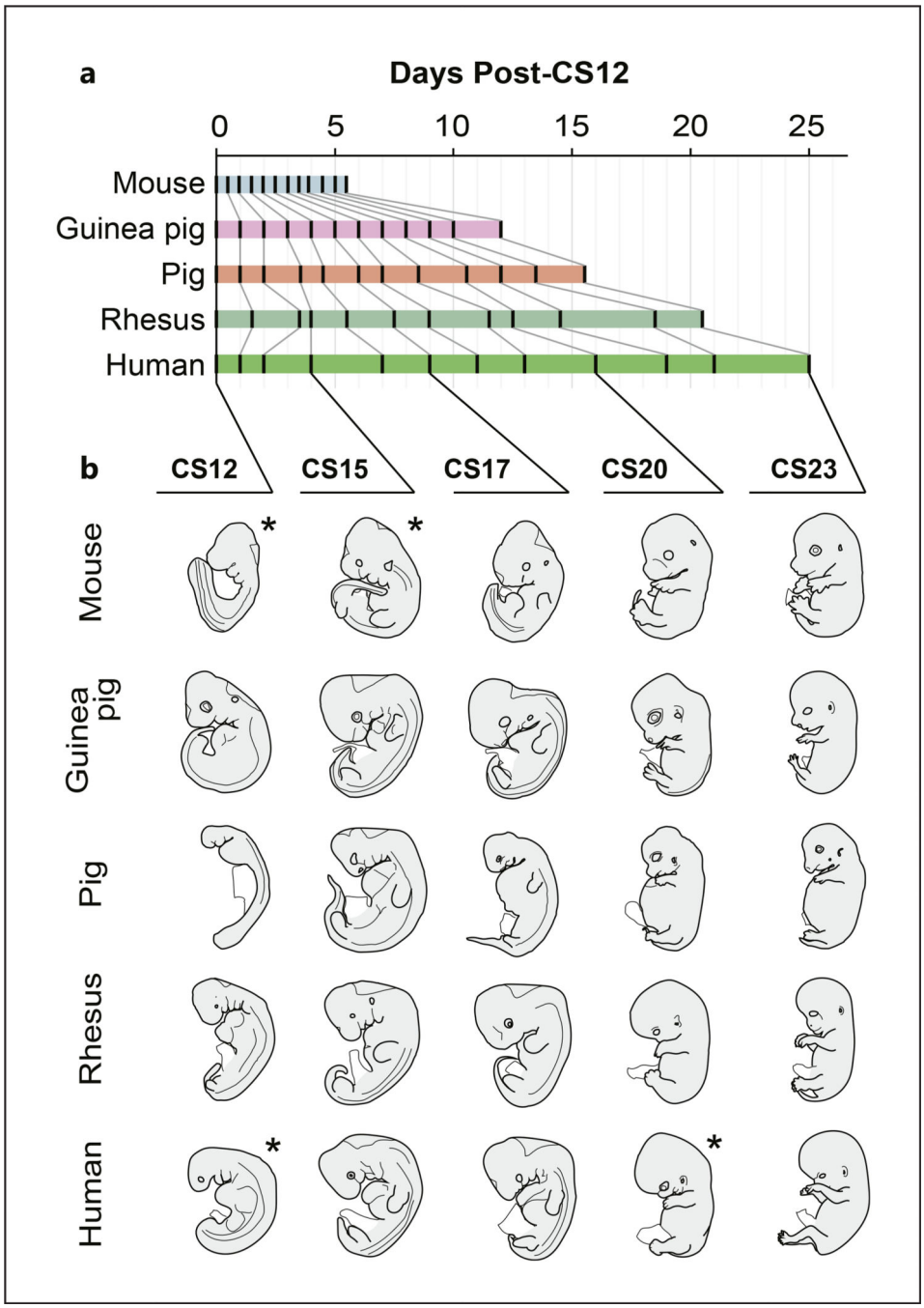

Fig. 3.

Variation in late stages of mammalian embryogenesis. **a** The duration of later stages of embryonic development (CS12–23) in five mammalian species, ranging from 6 days (mouse) to 25 days (human). Black lines indicate individual Carnegie Stages, and grey lines connect these stages across species. Each stage of embryonic development is extended in species with slower development (e.g., human). **b** Lateral-view outlines of select embryos in the same five species, redrawn from photographs in Butler and Juurlink [1987]. Stars

indicate that a source embryo photograph was mirrored along the vertical axis for visual consistency.

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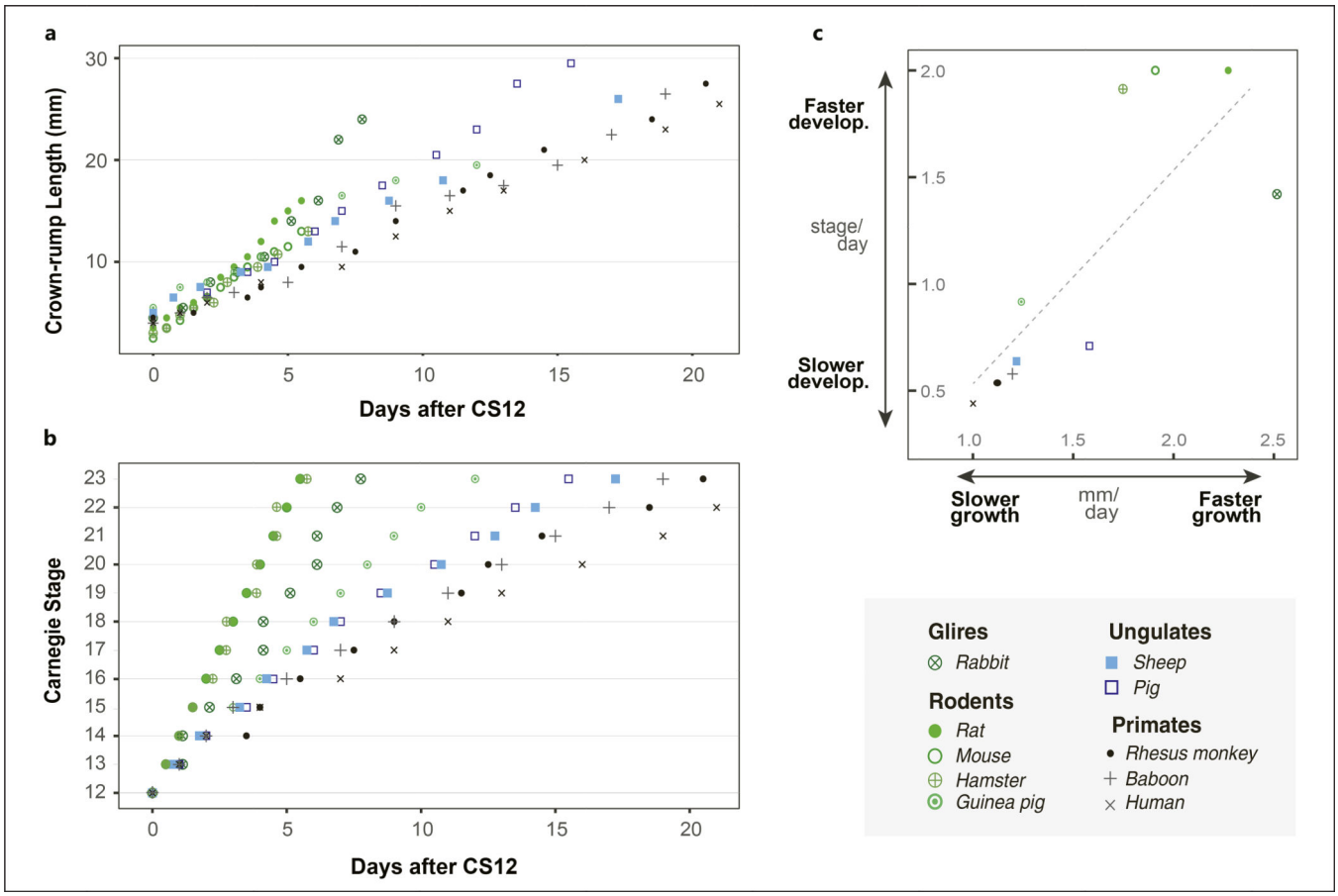

Fig. 4.

Variation in the pace of embryonic development and growth in mammals. **a** The rate of growth across species during the later stages of embryonic development (CS12>23), measured as the crown-rump length (CRL) in days post-CS12. Exceptionally rapid growth is observed in mouse and rabbit, and exceptionally slow growth is observed in primates. **b** Over the same period of late embryonic development (CS12>23), species also differ in the pace of development, here shown as the progression of Carnegie Stages relative to days post-CS12. Species that progress through developmental stages rapidly (e.g., mouse, rat, rabbit) also exhibit rapid growth (above); slow development (e.g., in primates) is associated with slow growth. **c** The average pace of embryonic development (*y*-axis: stage/day) is correlated with the pace of embryonic growth (*x*-axis: mm/day) during the later stages of embryonic development (CS12>23).

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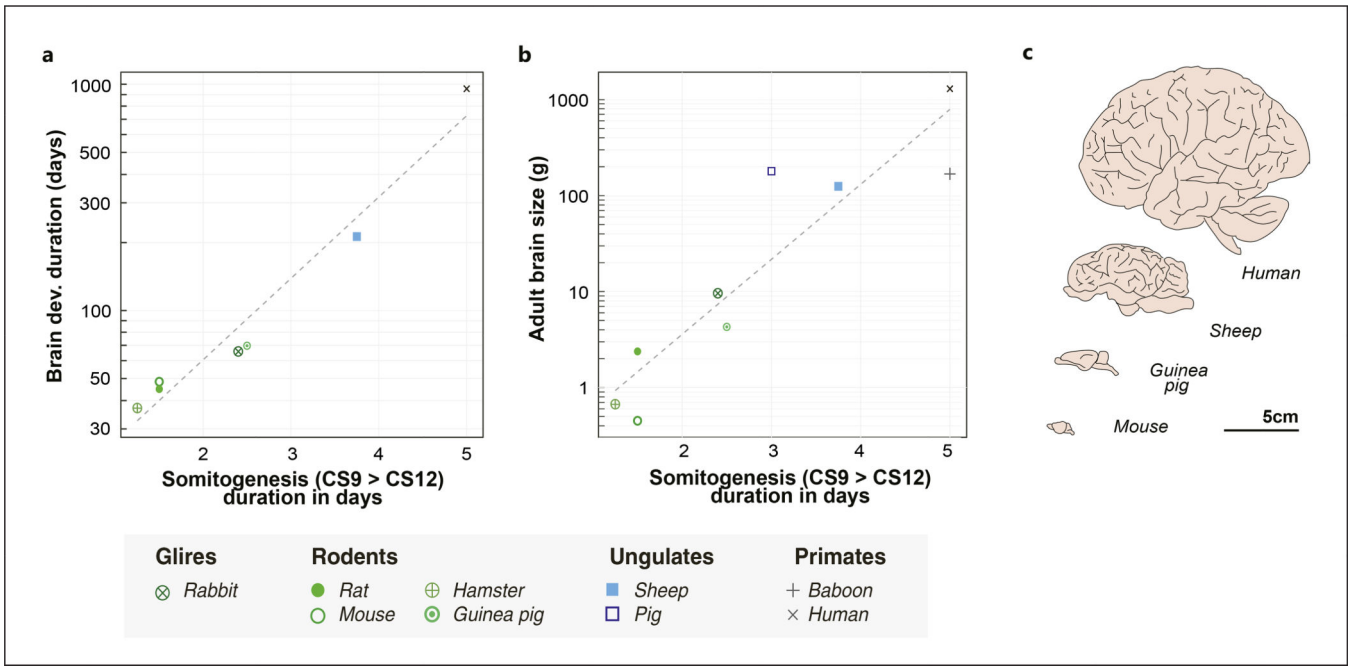

Fig. 5.

Two major variables in mammalian brain evolution – the duration of neurodevelopment and adult brain size – can be predicted by the duration of a somitogenesis. **a** Species with longer periods of somite formation during embryogenesis also have longer periods of brain development over the lifetime, suggesting a tight linkage between the pace of brain and body development. **b** Similarly, the duration of embryonic somitogenesis predicts adult brain size. The pace of neurodevelopment is linked to the pace of development in non-neural structures like somites, indicating the evolution of more global patterns of development.