Final author manuscript for Chapter 1 of *Vertebrate Embryogenesis: Embryological, Cellular, and Genetic Methods* (Methods in Molecular Biology 770), ed. Francisco J. Pelegri (New York: Humana Press), pp. 1–20. The final publication is available at http://www.springerprotocols.com/BookToc/doi/10.1007/978-1-61779-210-6.

Approaches and Species in the History of Vertebrate Embryology

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Abstract

Recent debates about model organisms echo far into the past; taking a longer view adds perspective to present concerns. The major approaches in the history of research on vertebrate embryos have tended to exploit different species, though there are long-term continuities too. Early nineteenth-century embryologists worked on surrogates for humans and began to explore the range of vertebrate embryogenesis; late nineteenth-century Darwinists hunted exotic ontogenies; around 1900 experimentalists favored living embryos in which they could easily intervene; reproductive scientists tackled farm animals and human beings; after World War II developmental biologists increasingly engineered species for laboratory life; and proponents of evo-devo have recently challenged the resulting dominance of a few models. Decisions about species have depended on research questions, biological properties, supply lines and, not least, on methods. Nor are species simply chosen; embryology has transformed them even as they have profoundly shaped the science.

Key Words: Developmental biology; embryology; evo-devo; history; methods; model organisms; species choice.

Running Title: History of Approaches and Species

1. Species Choice

Species choice has recently become prominent and controversial in debates over the pros and cons of the dominant "model organisms" in developmental biology ***(1)***. New systems seem to be announced almost monthly and laboratories are now more likely to cross species boundaries too. While this volume aims to promote that shift, this chapter puts these changes into historical perspective.

Embryologists have chosen organisms for their medical, agricultural, fisheries, sporting or other practical importance, or because they were considered biologically special. They have worked on surrogates for the species of most interest, especially humans, and on convenient representatives of groups ***(2)***. Different kinds of embryology have exploited various vertebrates in contrasting ways. Late nineteenth-century evolutionists, for example, risked life and limb on expeditions to hunt phylogenetically strategic embryos for histology. Twentieth-century experimentalists chose accessible organisms that would provide abundant living, easily analysed embryos on demand.

The histories of such models as chick, Xenopus, mouse and zebrafish show that species selection never simply matches research questions and biological properties. It is also about a community's values, institutions, networks and techniques: the kind of research it admires, the supply lines it can set up, the methods it can develop and, increasingly, the features it can engineer ***(3,4,5,6,7,8,9)***. So species

are not simply chosen for embryology. Complex experiments need elaborate infrastructures around highly domesticated organisms, but even to produce the most basic description embryos have to be seen within a developmental frame. It is easy to take this for granted today; historically, it was necessary to set up standard series and to challenge competing interpretations by other people ***(10,11)***.

Species choice creates opportunities and sets limits that strongly shape research ***(1)***. Competing research programmes invest in rival organisms ***(12,13)***; scientists bet on which organism–problem combination will prove most productive; agencies fund one rather than another. This is now clear for particular organisms and episodes, especially in the later twentieth and early twenty-first centuries, but the overall pattern is only starting to come into view. The chapter introduces the major approaches in the history of research on vertebrate embryos ***(14)*** and shows, in broad outline, why and how they have exploited different organisms. It begins to survey the long-term politics of species choice in embryology. (*See* **Note 1**.)

2. Histories of Development

Philosophers and physicians had for centuries investigated the generation of various animals and especially the chick, because its large eggs were abundantly available as food. But only in the age of revolutions around 1800 was embryology made a separate science. Developing embryos were framed as the objects of interest by rejecting older views, for example, of the acquisition of a rational soul as the crucial event in human pregnancy, and by using new techniques. Especially in German university institutes of anatomy and physiology, microscopists explored how complex bodies develop from simple beginnings. Through the mid-1800s they collected and dissected specimens, preserved them in spirits of wine, and observed and drew them through increasingly effective microscopes. They set up developmental series, correcting times for temperature where they could, and selecting representatives against which to assess new finds. They analysed embryos into germ layers and cells. Copper plates or lithographs accompanied the most prestigious publications (**Fig. 1**).

Medical and anthropological interest focused on humans, but anatomists had to rely on encounters with aborting women and the occasional post mortem. So embryos were inaccessible for about the first fortnight and rare for the next few weeks. Suspicions of abnormality made it hard to have confidence in accounts of normal development. Conveniently then, the most exciting comparative discoveries, such as the 1825 announcement of "gills in mammals" ***(16)***, reinforced the assumption that, across all the vertebrates, early development was fundamentally the same. So researchers fished amphibian spawn out of ponds, warmed hen's eggs in artificial incubators, and bought and bred rabbits and dogs. Physiologists criticized those who concentrated on human material they saw as uninformative. "[T]he history of the bird embryo is … the ground on which we march forward," while "that of the mammalian fetus is the guiding star, which promises us safety on our route towards the development of man" ***(17)***.

Yet embryologists also hoped that embryos would reveal the true relations between groups more clearly than in later life, and thus help comparative anatomy to produce a natural classification. To explore the play of difference within the underlying unity, they collected viper eggs, acquired deer from hunters, and obtained the conveniently transparent teleost embryos by artificial fertilization ***(5,18,19)***. Dealers supplied occasional exotics, and when Louis Agassiz emigrated from Switzerland to the United States in 1846 he opened up the American fauna, notably

fishes and turtles, for comparative embryology ***(20)***. Embryologists were few, though, and the biological, geographical and social obstacles were large. A major survey of 1881 still identified huge gaps ***(21)***—but by then things were beginning to change.

3. Ontogeny, Phylogeny and Histology

Darwinism drew on embryology for some of the strongest and most detailed evidence for common descent. From the late 1860s, with the slogan "ontogeny recapitulates phylogeny," the German zoologist Ernst Haeckel raised its profile in the universities and among the general public ***(22,23)***. He also changed its species politics. Nothing had been so damaging, he controversially declared, as concentration on the development of the chick. This had suffered such major changes from the ancestral form of the vertebrates—it was, in Haeckel's terminology, so "cenogenetic"—as to give a wholly misleading view. Embryology should start again should start again from the acraniate amphioxus and systematically pursue comparative research ***(24)***. While teaching focused on a few types, usually including the chick ***(25)***, he encouraged embryologists to discover the origins of the vertebrates, of tetrapods, and especially of human beings.

Land-locked European researchers, most of them pursuing careers as professors of anatomy or of zoology, created new institutions and exploited imperial networks to gain access to the rest of the world ***(26)***. Marine stations made it possible to utilize the sea more efficiently. Haeckel's student Anton Dohrn founded the most important in 1872 at Naples, where the Russian Alexander Kovalevsky had already influentially explored the development of ascidians and amphioxus and significant work on elasmobranchs would be done ***(27,28,29)***. Embryologists took advantage of an increasingly global web of collectors, for example, to establish a breeding colony of opossums, an American marsupial, in Bavaria ***(30)***.

The most intrepid scientists set sail to bring home "living fossils" and "missing links." They expected to find evidence of the major transitions most faithfully preserved in the early embryos of these groups. They caught lungfish spawn and other documents of tetrapod origins in South America, West Africa and the Australian bush ***(11,31)***, which also provided embryos of monotremes (egg-laying mammals): the platypus ***(26)*** and the spiny anteater or echidna. Colonial officials and settler farmers gave another Haeckel student Richard Semon access to echidna country and helped recruit native Australians. They staffed his camp and collected the nocturnal anteaters that lived, shyly and quietly, in the most impenetrable bush. Many settlers had never seen one, but the "incomparable nose and hawk's eye" of "the blacks" could follow the slight and complex tracks over difficult terrain to the hollows where the animals slept by day (**Fig. 2**). So they were cross when Semon paid little or nothing for the more numerous males ***(32)***. Many females were also sacrificed in vain. He had to preserve specimens on the spot, and because the aborigines returned at dusk, often ended up dissecting uterine embryos out of their tight-fitting shells "by the light of a flickering candle" ***(33)***.

The explorers valorized their own derring-do, and excused the gaps in their collections, by presenting rabbit breeding as tame ***(33)***. Yet another of Haeckel's students, Willy Kükenthal, accompanied whalers in the Northern seas, but found it hard to intervene during the freezing storms on the ships, where everything had to happen fast. He did better at the processing stations in Spitsbergen ***(34)***. The Erlangen Darwinist Emil Selenka's hunting trips to the East Indies laid the foundations of the embryology of apes. But he lost rare treasures in a boat collision and was so sick with malaria that his wife Lenore had to make good the loss ***(35)***. The most arduous, and

among the least successful, embryological collecting was of emperor penguin eggs during the fateful "winter journey" of Robert Falcon Scott's Antarctic expedition in 1911. Working out the embryology of "the nearest approach to a primitive form not only of a penguin, but of a bird" had seemed "a matter of the greatest possible importance," and cost biologist Edward A. Wilson his life, but sadly, no one much cared about the three fairly late-stage eggs that made it back ***(36,37)***.

Collecting worked profound intellectual transformations. This is because it framed materials as embryos that the suppliers had often interpreted in other terms. The aborigines knew how to track the echidna, or "cauara," because it was a prized delicacy; they also told Semon of its origin from a bad man who was filled with spears. He impressed "the bushmen" by showing that the young were not "conceived on the teat," as they had believed, but began, like other mammals, in the womb ***(32)***. Some of the deepest transformations went on closest to home. Even women who knew they were pregnant—and in the early stages, especially before hormonal tests, many did not—rarely interpreted the blood clots they passed in embryological terms. Depending on whether or not a woman desired a pregnancy, she might think in terms of a child to come or of waste material that had to be removed. Anatomists appropriated bleeds that had been experienced variously as unremarkable late periods, distressing miscarriages or desired restorations of menstrual flow ***(10)***.

Embryos of different species were then made equivalent by analysing them in comparable ways. The great innovation of the 1870s was routine serial sectioning with microtomes to give more detailed access to internal forms than dissection could achieve. Though embryos were sometimes observed fresh using low-power microscopes and drawing apparatus, sectioning became central to embryological technique. Once obtained, and sometimes cultured, the material was fixed and stained, embedded and cut by methods adapted to each taxonomic group and stage ***(38,39)***. For particularly complex forms it became common to reconstruct three-dimensional views from the sections, either graphically or in wax ***(40)***.

Debates over evolution made degrees of similarity and difference so contested that other vertebrates could no longer stand in for human embryos. Haeckel's leading critic, the Swiss anatomist Wilhelm His, reformed the field by applying the microtome to a rich supply of precious human specimens from the third week to the end of the second month. Since he could not set up rigorous stages for this scarce and variable material, he invented a 'normal plate' that simply arranged representative specimens in series ***(10)***.

Anatomists now prided themselves on studying human embryos directly. In 1914 they established this non-evolutionary human embryology, primarily using material recovered during surgery, by founding the Carnegie Institution of Washington Department of Embryology at the Johns Hopkins University ***(41,42,43)***. A primate colony was installed there in the 1920s ***(44)***. (Today the human embryo collection is at the National Museum of Health and Medicine in Washington, D.C.)

Meanwhile, as evolutionists increasingly questioned Haeckel's doctrine of recapitulation, high-profile disagreements sent the field into crisis ***(11,22,45)***. To reassess the relations between ontogeny and phylogeny, the German anatomist Franz Keibel organized an international series of 16 vertebrate normal plates ***(11)*** (**Fig. 3**). The revived comparative studies were institutionalized in 1911 in the International Institute of Embryology. Constituted through a series of meetings in different locations, this club promoted 'salvage' embryology: collecting endangered colonial mammals for what became the Central Embryological Collection at the Hubrecht Laboratory in Utrecht ***(47)***. (It was transferred in 2004 to the Natural History Museum

in Berlin.) Evolutionary embryology nevertheless declined after World War I, and experimentalists disparaged comparative work as merely "descriptive."

4. Experimental Cultures

From the 1880s, some embryologists took a radically different approach, reconstructing embryology not as a historical science but on the model of the new experimental physiology with its ideal of controlling life. Occasional earlier experiments had generated additional forms to anatomize and taxonomize, but now the focus was less on evolutionary questions than on how, in the present, one stage produced the next.

The anatomist Wilhelm Roux and other exponents of "developmental physiology" or "developmental mechanics" employed a range of interventions, mechanical (shaking, cutting, constricting, pressure, gravity, centrifugal force), thermal, chemical and electrical. The pioneers tended to use small metal scissors, needles and knives; in the next generation zoologist Hans Spemann's microsurgery relied on hair loops and much finer glass instruments that he made himself ***(48)*** (**Fig. 4**). The new stereomicroscopes allowed finer manipulations ***(50)***, but careful culture was at least as important as fancy apparatus, especially since antibiotics came in, for the more challenging cultures, only after World War II. Keibel's elaborate normal plates were condensed into diagnostic "normal stages" ***(11)***. "Fate maps" used vital dyes to show what early regions would become ***(51)***. Grafts were also marked by species differences in pigmentation.

Species here mattered little for their own sakes. So fishes tended to lose out, because researchers no longer much cared about either their extraordinary diversity or their position as basal vertebrates, while other classes provided living embryos that were more easily cultured and manipulated in large numbers ***(5)***. Among the vertebrates the freely accessible, large and extremely resilient eggs of local Amphibia were much the most popular for extirpation, explantation and transplantation, with chicks in second place ***(49,52,53)***. Relevant work on mammals went on in the new field of reproductive science ***(54)***. The pig was used in teaching alongside the chick.

Embryologists had always specialized in certain groups, but never as much as Spemann, co-discoverer of the organizer. He arranged his career and those of almost all his students and collaborators around microsurgical work on species of the salamander Triton (now mostly Triturus). This concentration shows the shape of things to come, but the breeding season still limited the experiments to the spring ***(55,56)***.

5. Model Organisms

After World War II, massively expanded government funding allowed biological and especially biomedical research to expand and intensify. Seeking the most productive experimental systems, biologists and especially geneticists focused on a few readily available model organisms. With their short generation times, small adult sizes, and general suitability for laboratory domestication, these species would dominate research on development.

Evolution was sidelined as the new "developmental biology" studied cellular, molecular and genetic processes, and increasingly patterns and mechanisms of gene expression, in the most convenient organisms. Comparative research continued in traditional departments, museums, marine stations and fisheries labs ***(57)***, and experiments used a wide variety of embryos *(see* **Note 2***)*. But just a few species account for most of the big growth in developmental biology ***(58,59)***. The fruitfly

Drosophila melanogaster and later the nematode Caenorhabditis elegans bid most strongly to become the embryological E. coli, but three and then four vertebrates were among the top half-dozen species, in part because of their medical relevance, in part because they were more suitable for experimental embryology and biochemistry.

The most venerable, the chick, was used in the postwar era especially to explore the development of limbs and nerves ***(60)***. Much research on the neural crest has employed chick–quail chimeras, with their histologically distinguishable nuclei as intrinsic markers ***(61)***. Chick eggs may have been exploited for embryology before other sciences, but more often developmental biologists adopted species that had already entered laboratories. Introduced in the 1930s as a test animal for pregnancy diagnosis, the South African clawed frog Xenopus laevis has large eggs and—the basis of the test—an injection of chorionic gonadotrophin will induce laying almost at will. By releasing experimenters from the seasonality of indigenous amphibian spawning, this increased productivity and marginalized other species. Xenopus was soon favored for combining experimental embryology with biochemistry and later molecular biology, but the genetic possibilities of this pseudotetraploid species were limited ***(7)***.

Most significant for medicine and agriculture was the opening up in the 1960s of preimplantation mouse embryos for culture and manipulation ***(62,63)***. While the larger rabbit had been preferred for work on fertilization and embryo transfer before World War II, the more general establishment of inbred mice as standard genetic models for human beings ***(8)*** gave them a decisive advantage (**Fig. 5**). By the 1980s more articles in developmental biology journals were devoted to mice than any other species ***(59)***.

"The mammalian embryo" tended to mean "the mouse," but researchers had strong practical incentives to cross species barriers. Embryo transfer in livestock was made a major industry in the 1970s ***(65)***, and human embryology and reproductive medicine were revolutionized with the 1978 achievement of a live birth following in vitro fertilization ***(66,67)***. Some innovations, notably freezing and cloning by nuclear transplantation, were first achieved with the larger embryos of sheep ***(62,68)***. Interest in exotics was initially rare, but vets and zoos have been engaged in reproductive science for several decades ***(69,70)***. With echoes of the International Institute of Embryology, cloning is being controversially applied to conserving endangered species ***(71,72)***.

Organisms had long needed work to adapt them for embryology, if only in the form of normal plates and/or special methods for culture or histology. As scientific objects, they were always made as well as found. Now they were increasingly heavily engineered for research in developmental genetics and cell biology, with mutant stocks and a panoply of sophisticated techniques for following and manipulating cells and gene expression. Most revolutionary was the combination since the 1970s of the new molecular cloning with older methods of genetic screening and embryo manipulation ***(4)***. The investments of individuals and groups combined with the laboriously-built-up advantages of resources, techniques and colleagues to entrench model systems. These powerfully channeled research to the questions they were best suited to answer. Distinct communities specialized in different organisms, procedures and phenomena.

A new model could be successfully launched only with the prospect of greater productivity and high-level support to achieve it within a reasonable time. This happened in the 1980s for the zebrafish Danio rerio. A pet-shop staple had in the 1970s been turned into an effective genetic organism that could be screened much

faster than mice and developed in full view. By the 1980s its potential for combining genetics, experimental embryology, neuroanatomy and cell-lineage analysis was clear. A research community was becoming established, when in the late 1980s senior Drosophila developmental geneticists alighted on the zebrafish as the most suitable vertebrate for the mass mutagenesis that had proved so transformative in flies. The results of a "big screen" in Tübingen and Boston, published in 1996, stimulated major investment by the NIH ***(9,73)***.

Model organisms were never the whole story. Some developmental biologists insisted through the 1970s and '80s on studying unfashionably difficult vertebrates, such as various fish, urodeles, turtles, crocodilians and marsupials; some models were only locally important, for example, the teleost medaka in Japan. Things changed more profoundly when new approaches generated new questions and new methods made innovation easier.

6. Beyond Models?

From the 1990s the dominance of the few big embryological species was challenged in various ways, but these remain firmly ensconced and have in some ways become even more attractive. New organisms are emerging, while the old survive by being re-engineered and reconceived.

The discovery of deep molecular homologies across phyla breathed new life into studies of development and evolution that had continued through the twentieth century but most developmental biologists had scorned. Evolutionary developmental biology ("evo-devo") claims to revive and revise Haeckel's questions at the molecular level ***(74)***. In evo-devo, and "eco-devo" or ecological developmental biology, species politics are more explicit than ever. Proponents critiqued over-reliance on model organisms on the grounds that precisely the qualities that had led to their selection, notably rapid, strongly canalized development that was resistant to environmental effects, made them unrepresentative of their own taxa, not to mention life beyond the laboratory walls ***(75)***. Funding the old models would just privilege the old reductionism, leaving evolution and ecology out of account. Conveniently, whole-genome sequencing and powerful new methods of functional analysis lowered the barrier to comparative studies.

The stakes are high as the NIH favors established models and the NSF promotes new ones ***(1)***. Defenders of old systems fight for continued recognition—one even wrote of "'anti-chick' racism" ***(60)***—while reformers advertise their favorite organisms and debate selection criteria. The dog, with its enormous selected within-species variation, has been proposed as a model for studying evolutionary changes in regulatory genes. The contrast between eyed surface and eyeless cave-dwelling forms of the Mexican tetra Astyanax mexicanus is advocated as a model for evolutionary response to environmental variation (**Fig. 6**). These choices highlight conceptual themes, rather than simply picking diverse leaves from the phylogenetic tree ***(77)***.

Evo-devo and eco-devo were initially critical of models, but may accept them if reframed as organisms in their evolutionary and environmental contexts. Established models are even being repositioned not as sufficient surrogates for the rest of the animal kingdom, but as beachheads from which to explore phylogeny and ecology ***(1)***. Whether primarily oriented towards physiology, evolution or ecology, or trying to integrate all three perspectives, developmental biologists today share key methods.

In laboratories devoted to physiological mechanisms of development, the traditional models, with their better-developed genome databases and stock centers,

are also being enriched. On the one hand, more can now be done in any one species. Transgenic technology, for example, which initially only increased the genetic advantages of the mouse, has finally made it easier to do reverse genetics in frogs and chicks. For the former this has involved international cooperation to build resources for the previously little-used Xenopus tropicalis, a close relative of X. laevis with a shorter generation time and smaller, diploid genome ***(78)***. On the other hand, as several chapters in this volume show, researchers have in the last decade become more flexible and adventurous about using multiple species in any one project.

7. Conclusion

Scientists with different approaches have adapted different species for embryology. The most dramatic contrast is around 1900. Comparative evolutionary embryologists still traveled the world to obtain lungfish, echidna and apes, while developmental physiologists already devoted whole careers to experimenting systematically on the local amphibians. What is convenient for one kind of work may also suit another; Darwinists had previously dissected, sectioned and modeled those same frogs and newts. But though existing knowledge and arrangements favor continuity, when much else is in flux long traditions are as remarkable as change. They depend on finding fresh advantages and withstanding new competition. Take the grand old man of embryological species, the chick. In 1835 Valentin advocated its use in preference to rare and often abnormal human specimens, but forty years later Haeckel rejected it as phylogenetically misleading; it still played a significant role in teaching and as an experimental species, but recent defenders have had to fight for its privileged place in developmental biology.

The history of human embryos and their substitutes shows particularly clearly the play of continuity and change. Early and mid-nineteenth-century embryologists mostly studied chicks and domestic mammals as surrogates, and also as more general representatives of vertebrate development. By contrast, post-Darwinists prided themselves on researching human embryos directly, exploited the rise of operative gynecology to investigate ever earlier stages, and even modeled studies of other mammals on the human work. Early developmental biology tended to ignore human embryos as experimentally intractable, while engineering the mouse as the principal "model for man." Experiments with this and other laboratory species made possible in vitro fertilization, which brought human embryos into laboratory and clinic. For some techniques they again led the way.

The range of actively-researched species has varied a good deal. So has the rate at which new organisms have been domesticated for embryology and the height of the barriers between them. The chances of taxonomic innovation and of transfer between species, into as much as within embryology, depend on the perceived balance between difficulty and rewards. Obtaining scarce material from distant lands presented nineteenth-century comparative embryologists with a major challenge, even as improved transportation shrank the globe. But it could make a reputation, and a little tinkering was usually enough to adapt standard histological methods. From the 1930s, pregnancy testing and genetics provided experimentalists with improved frogs, mice and later fish, which developmental biologists then customized with specific methods and resources. By the 1970s and '80s, problems, techniques and resources seemed so segregated that the vast majority stuck to the model in which they had trained. Exemplary work on Drosophila and the universalizing effects of molecular cloning brought the field together. In the 1990s, more transferable methods and the prospect of tackling new (and old) questions opened things up. But species preference

is no simple cost-benefit calculation; it has an aesthetic dimension too: with what animals, and what other humans, does an embryologist wish to spend time?

8. Notes

1. For general references on the history of embryology, see **ref. *14***; those given here are limited to the historical writing, or, where this is unavailable, selected primary sources, most relevant to questions of species and methods. The chapter does not attempt to explore the effects of species choice on embryological knowledge.
2. For the range, see the research topics and the "'Supply and demand' service for laboratory animals" listed in the Hubrecht Laboratory's *General Embryological Information Service*, which ran from 1949 to 1980.

Acknowledgments

For comments on a draft I thank Scott Gilbert, Martin Johnson and Francisco Pelegri. Research was supported by the Wellcome Trust [074298].

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Figures

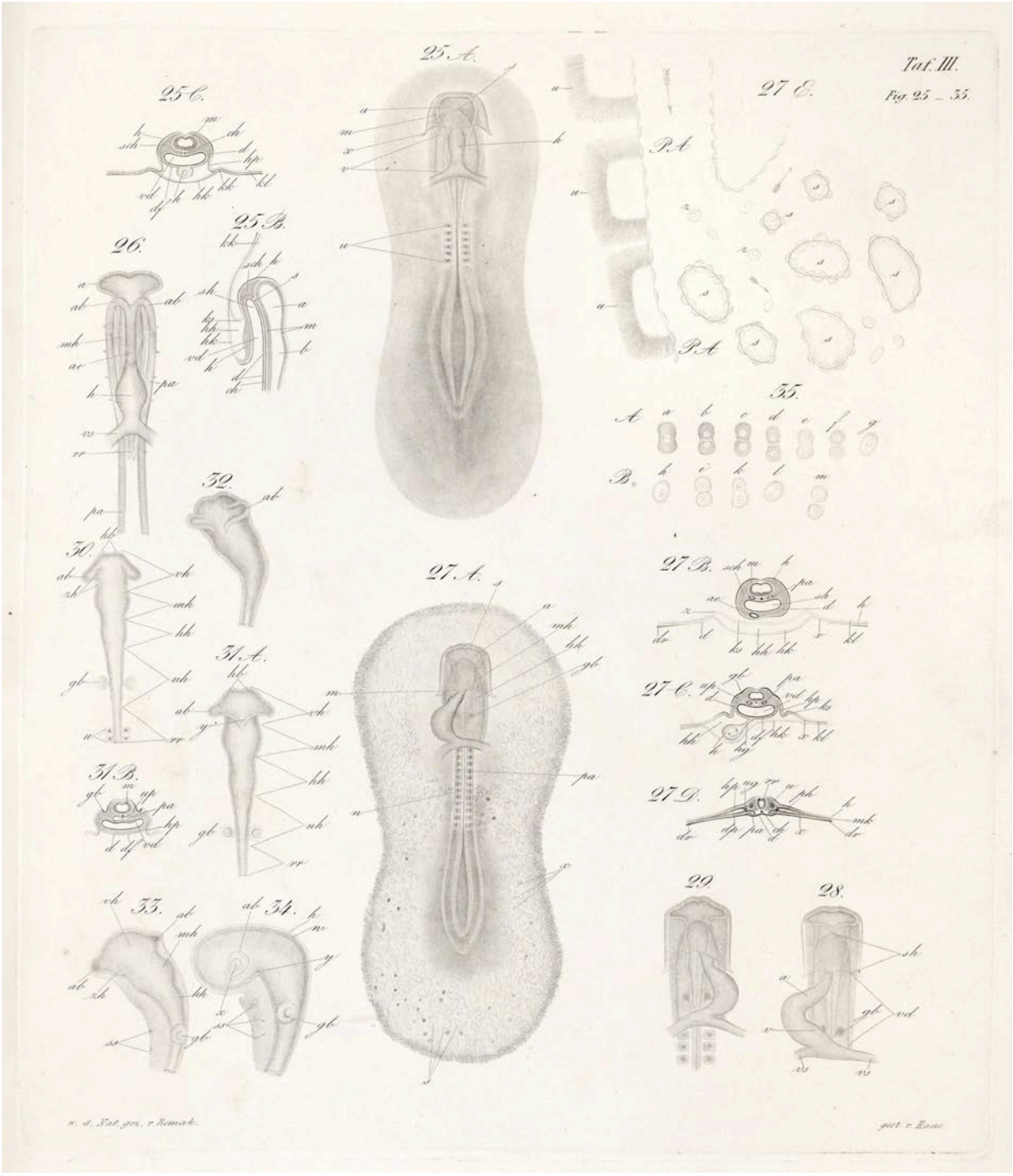

Fig. 1. Copper plate, showing whole views, organs, sections and (blood) cells of second- and third-day chick embryos, from the 1855 book by the Berlin microscopist Robert Remak that refined the germ-layer doctrine and linked it to the cell theory. He argued that division of the egg cell produced layers composed of cells that each divided and differentiated to produce specific tissues and organs. Engraving by Haas after Remak's own drawings, reproduced from **ref. *15*** by kind permission of the Syndics of Cambridge University Library. Printed surface 30 x 26 cm.

Fig. 2. The German zoologist Richard Semon's echidna hunters and their prey. (A) Semon's "particular friends," Ada and her husband Jimmy, in the camp on the River Boyne, a tributary of the Burnett, in Queensland. Though Semon regarded the aborigines as "one of the lowest human races," he admired their skill in the noble art of hunting. Jimmy, once a famous warrior, was Semon's "best huntsman" and a fine raconteur. The bottles on the table were likely for preserving and staining the embryos. (B) An echidna, or spiny anteater. Reproduced from **ref.** ***32*** by kind permission of the Syndics of Cambridge University Library.

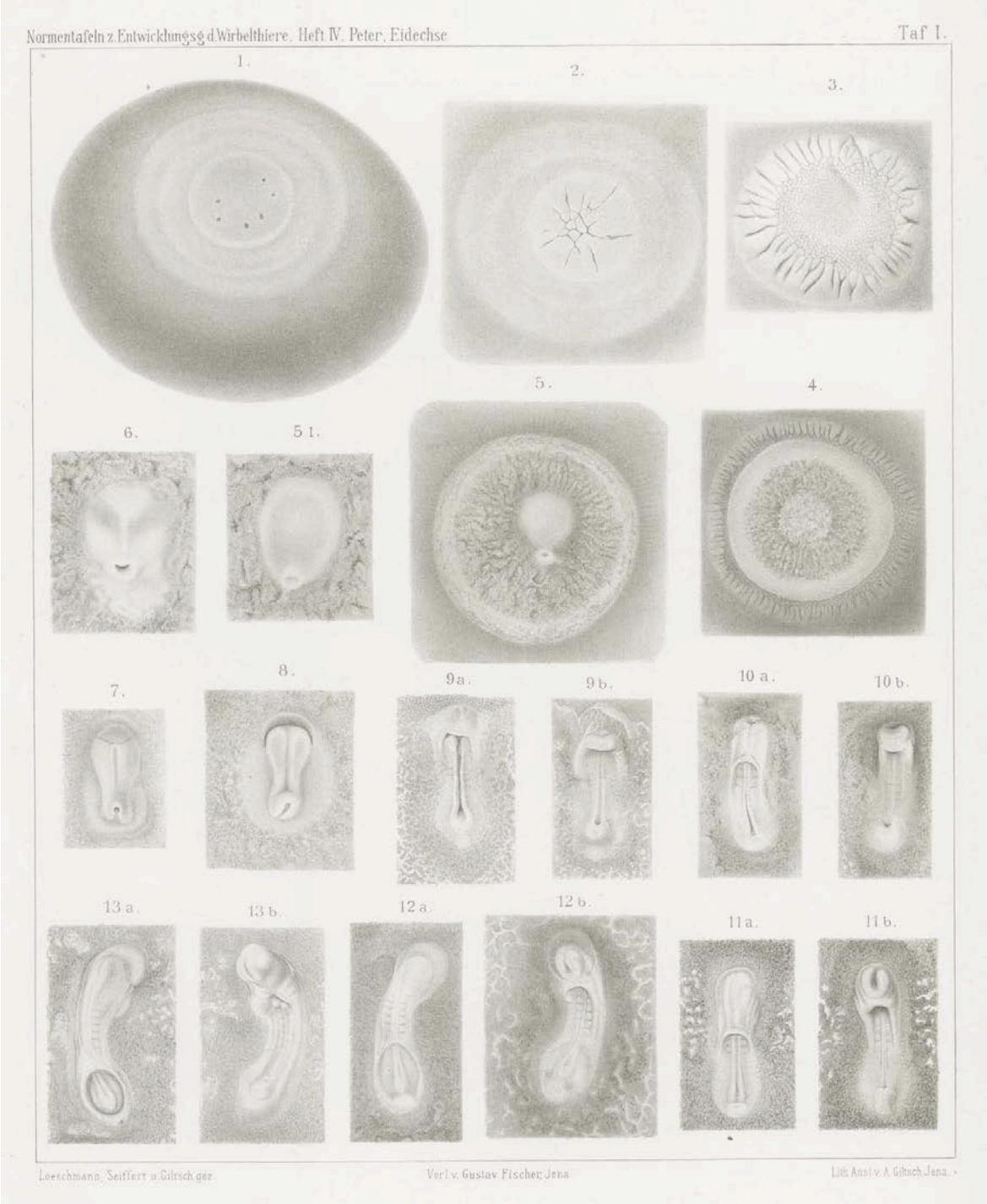

Fig. 3. The normal development of the sand lizard, Lacerta agilis. The fourth (1904) volume in Franz Keibel's *Normal Plates on the Development of the Vertebrates* is the only one devoted to a reptile, though he had hoped to include turtles too ***(11)*** and snakes and crocodilians had also been studied before. The author, the Breslau (now Wrocław) anatomist Karl Peter, raised the lizards in terraria over six summers. This first of four plates covers development from an uncleaved egg; magnification is 10 x (1–5) or 20 x (5^{I}–11); embryos 9–11 are shown from (a) dorsal and (b) ventral sides. Specimens were drawn unstained, with reference also to stained specimens and photographs, each drawing combining features that could be seen in nature only by illuminating from various angles. Lithograph by Adolf Giltsch, after drawings by Emil Loeschmann, a Mr Seifert and Giltsch, from **ref. *46***. Original dimensions of border 26.5 x 21.9 cm.

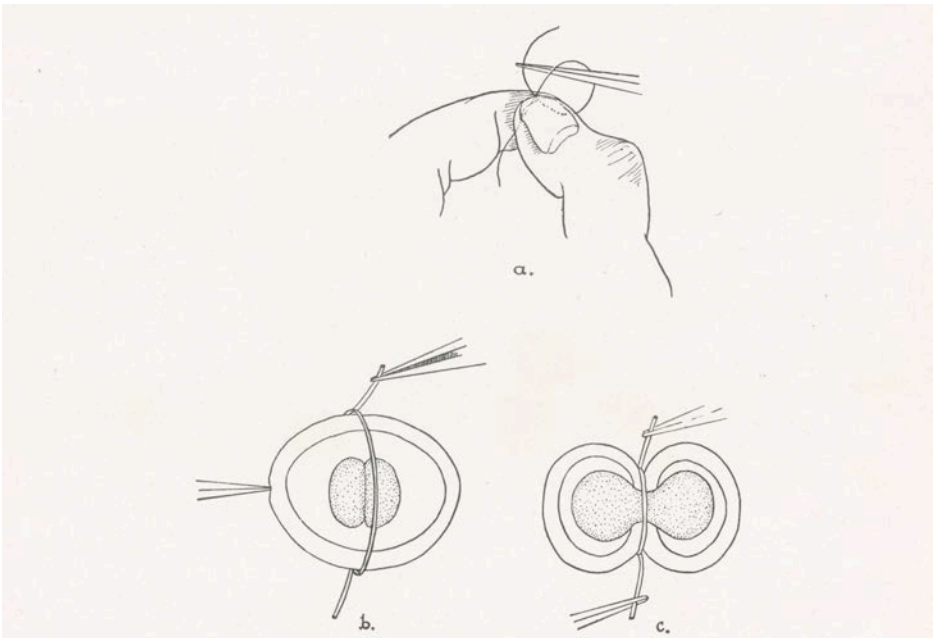

FIG. 19.—Constriction of a urodele egg (2-cell stage) with a hair. *a*=preparation of the hair loop; *b*=placing of the egg in the loop; *c*=constriction.

Fig. 4. "The production of twin embryos and of duplications in Urodela by constriction. After Spemann." Diagram from a manual for student practicals by Spemann's former colleague, Viktor Hamburger. Constricting only slightly produces conjoined anterior duplications. Spemann had used his daughter's hair. Hamburger recommended the Eastern newt, Triturus (now Notophthalmus) viridescens. Reproduced, by permission of the University of Chicago Press, from **ref. *49***.

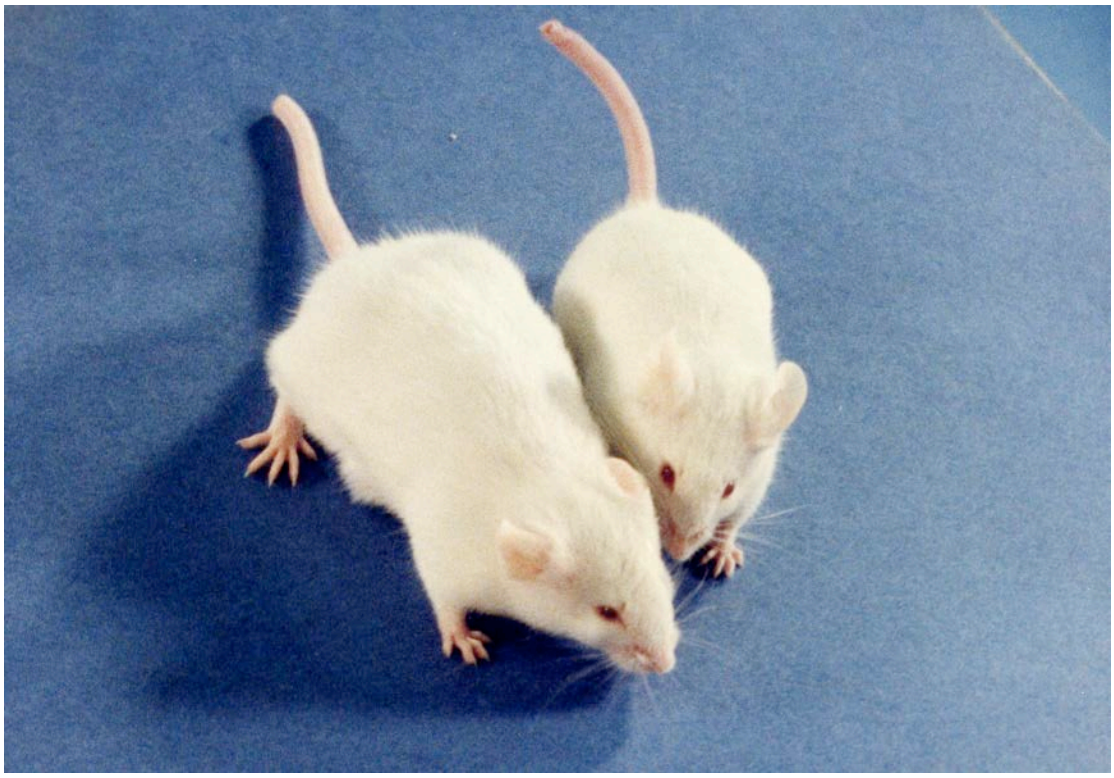

Fig. 5. A transgenic mouse. This image appeared on the cover of *Nature* in 1982 with the caption "Gigantic mouse—from eggs injected with growth hormone genes" (**ref. *64***). One littermate had a body weight almost twice that of its sibling because it carried a hybrid gene containing the mouse metallothionein-1 promoter fused to the rat growth hormone gene. Courtesy of Ralph L. Brinster, School of Veterinary Medicine, University of Pennsylvania.

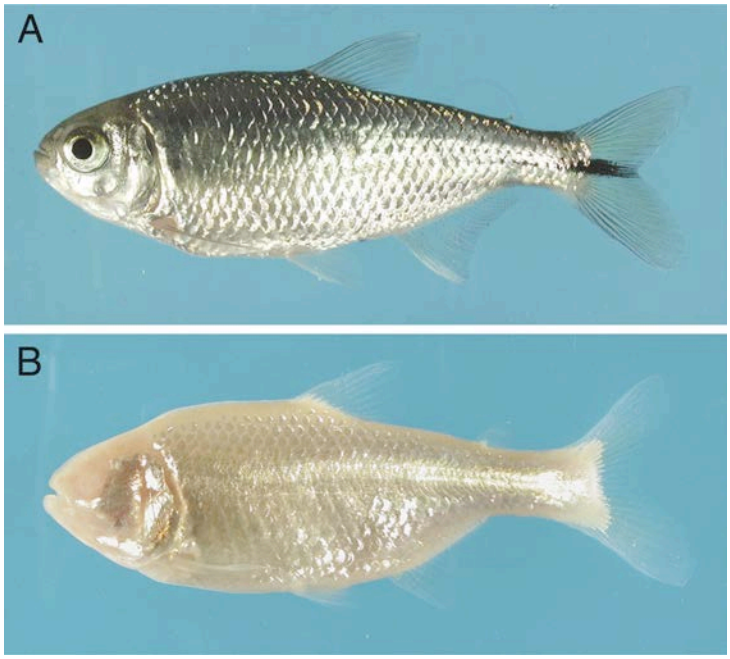

Fig. 6. The Mexican tetra, Astyanax mexicanus, a model for evolutionary response to environmental variation (see **ref. *76***). (A) Eyed surface fish, and (B) blind cavefish. Courtesy of William Jeffery, Department of Biology, University of Maryland.