Integrating regenerative biology with developmental psychobiology to understand behavioral recovery

Justin A. Varholick*
Department of Biology, University of Florida, FL, USA

*Correspondence should be addressed to justinvarholick@ufl.edu

Abstract

Developmental psychobiology (DPB) is a sub-discipline of developmental biology investigating behavioral development. Regenerative biology is also a sub-discipline of developmental biology, studying how tissues and organs heal and regenerate after injury. One aspect of healing and regeneration is behavioral recovery, involving the nervous system and coordinated movements. Behavioral recovery is often a secondary measure in many regeneration studies, primarily focusing on molecular and cellular mechanisms. Integrating regenerative biology with DPB would provide a basis for behavioral research on regenerative systems as a separate biological question to increase our understanding of behavioral recovery and the underlying role behaviors can have on the regeneration process. Here, I introduce three general principles of DPB: probabilistic epigenesis, “development from,” and Tinbergen’s four questions. I elaborate on how these principles reveal gaps in our knowledge concerning regeneration.

Introduction

Regenerative biology aims to understand how regenerative-competent animals regrow tissues and organs without scarring. For example, how does a salamander regrow a limb after amputation? After over a century of studies, we have made significant strides in understanding the processes underlying limb regeneration. However, we still face substantial gaps in our knowledge, such as understanding the recovery of limb behavior after injury. This is a crucial area of research, as understanding how behaviors recover after injury is essential for successfully applying regenerative biology in human medicine. Integrating developmental psychobiology (DPB) into regenerative biology could be crucial because functional recovery/regeneration is as necessary as structural recovery/regeneration. Support for this integration of disciplines is the focus of this manuscript.

DPB is a subdiscipline of developmental biology that integrates psychology to understand how behaviors develop[1–4]. Some relevant research topics include the development of animal behaviors (e.g., bird song, courtship displays, social attachments, food preferences) and human behaviors (e.g., reaching, grasping, walking, language, and learning). To understand the development of these behaviors, DPB takes a systems approach. It investigates the contribution of physiological, biomechanical, and environmental processes underlying behavioral development. All contributing aspects of these three underlying processes hold equal weight and coact as a team, much like the area of a rectangular prism depends 100% on the length, width, and height. Traditional dichotomies from other fields, like gene-environment, innate-acquired, nature-nurture, and hardwired-plastic, are traditionally disparaged by DPB researchers. From the DBP perspective, the development of a phenotypic trait results from the contributions of various physiological, biomechanical, and environmental processes. The goal of DPB research is to identify and specify how each of these processes contributes to the developmental expression of any behavioral attribute/trait. This is an important perspective because DPB allows us to understand all processes involved during each developmental phase to understand the induction, transformation, and maintenance of any behavior. Of course, such research can be cumbersome. Thus, individual DPB scientists typically investigate subsets of these processes while acknowledging that other unstudied processes continue contributing to the development of the behavior.

In this manuscript, I propose that general principles from DPB can help us understand how the regeneration of a vertebrate limb—or any other regenerative process—recovers function/behavior. Also, the perspective of DPB will focus attention on some ignored processes underlying regeneration because behaviors can play an active role in regeneration, similar to molecular and cellular signals. To elucidate the value of DPB for regenerative biology, I will describe three general principles from DPB: probabilistic epigenesis, “development to” versus “development from,” and Tinbergen’s four questions. I will briefly use each of these principles within the scope of DPB and then apply them to studies in regenerative biology to show their value in addressing some knowledge gaps. The aim of proposing the integration of DPB with regenerative biology is to stimulate research on these knowledge gaps to enhance our understanding of regenerative biology and promote the successful application of regenerative biology in human medicine.

Probabilistic Epigenesis

Probabilistic epigenesis is a framework proposed by Gilbert Gottlieb[5,6] that encompasses the coactional perspective employed in DPB. It was expanded and simplified from the developmental theories of the embryologist Paul Weiss[7] and population geneticist Sewall Wright [8], among other DPB scientists like Zing-Yang Kuo, T.C. Schnierla, and Daniel Lehrman[9]. It begins with a critique of predetermined epigenesis, which states that DNA codes for RNA, RNA codes for proteins, proteins lead to structures, and structures lead to function, activity, or experience. Simply, DNA → RNA → protein → structure → function. Instead of unidirectional effects, probabilistic epigenesis proposes bidirectional effects. Simply, DNA ↔ RNA ↔ protein ↔ structure ↔ function. Thus, instead of a unidirectional view of DNA serving as a blueprint for structure and function, the bidirectional view proposes that functions, experiences, structures, proteins, RNA, and genetic activity interact equally to make a phenotypic trait.

Modern molecular biology, epigenetics, and ecological developmental biology strongly support the coaction of multiple systems. They broadly indicate that each system is inseparable from the organism's development and directly affects one another. For example, incubation temperature affects sex determination in reptiles[10], gravity affects body axis formation in Xenopus[11], and monozygotic identical twins reared apart can have significant phenotypic differences[12]. At the time, incubation temperature, gravity, and other normally occurring aspects of the environment were frequently neglected when considering the role of genetic activity. Thus, Gottlieb proposed that they should be incorporated into research on behavioral development. To do so, he provided a framework of probabilistic epigenesis (Fig. 1). Here, genetic activity, neural activity, behavior, and environmental influences have coactional effects on one another throughout an individual's development. They each also have an equal effect on development. Thus, gene activity is equal to the role of the environment and behavior.

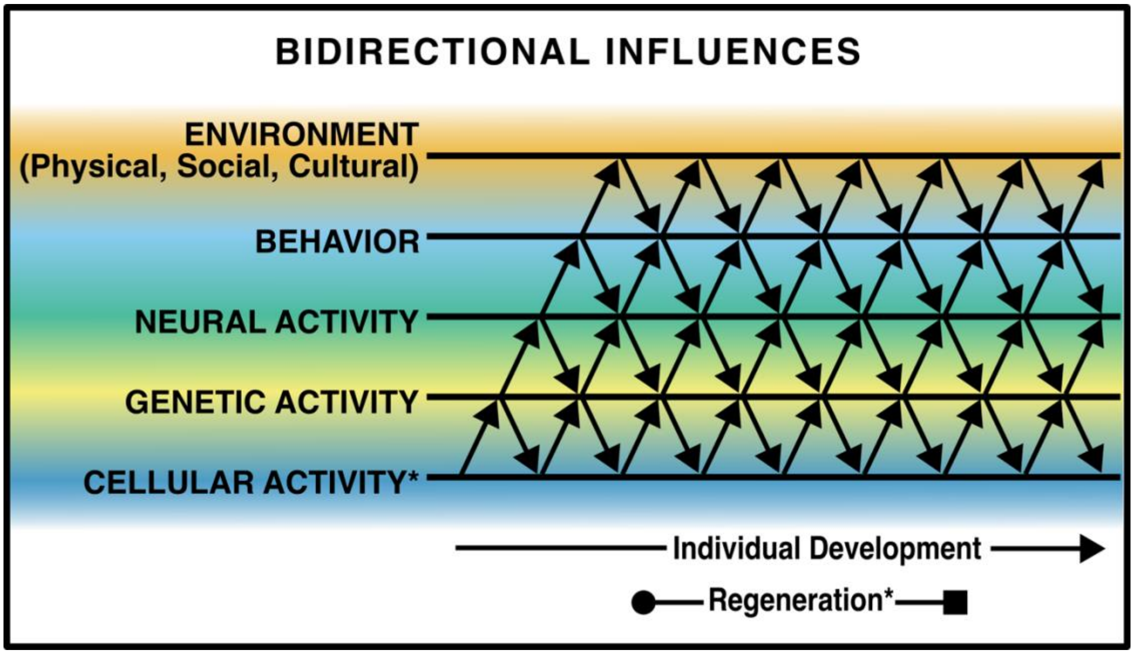

Fig. 1 Bidirectional influences in probabilistic epigenesis and regenesis. This is a modified framework of the one proposed by Gilbert Gottlieb (2) that can be applied to regeneration. The asterisks denote that cellular activity and regeneration have been added to the original framework. Each factor of environment, behavior, neural activity, genetic activity, and cellular activity interact across individual development and regeneration. Note that development continues with a directional arrow. At the same

time, regeneration has a designated initiation from the injury (denoted by the circle) and an ending when regeneration is theoretically complete (denoted by a square). The listed factors are not exhaustive.

Evidence of the probabilistic epigenesis framework already exists within regenerative biology. Much like development, the regenerating tissue uses DNA to make RNA to make proteins, make structures, and restore functions over time. Moreover, current biology recognizes that many external and internal factors can affect gene expression in a reciprocal, bidirectional, or coactional manner. The effect of crowding on zebrafish heart regeneration nicely demonstrates these reciprocal actions[13]. Zebrafish were exposed to one hour of crowding (i.e., ten fish per 250ml) daily for 30 days after ventricular cryoinjury to the heart. Only 30% of those exposed to crowding regenerated their hearts, while 100% of the control condition regenerated their heart. Thus, the environment has a direct effect on the regeneration of structures and subsequent functions (i.e., environment → structure and function).

This failure to regenerate was linked to a two-fold reduction in cardiomyocyte proliferation. Follow-up studies with the stress hormone agonist dexamethasone suggested that increased levels of stress hormones were a driving factor in the reduced cardiomyocyte proliferation. Thus, the environment affected hormone release, altering cell proliferation during regeneration (i.e., environment → hormones → cell proliferation). RNA-sequencing analysis showed that three genes were downregulated: *ankrd9*, *nr4a1*, and *igfbp1b*. This demonstrated that the environment also affected gene expression (i.e., environment → gene expression). Follow-up studies also suggested that the stressed zebrafish maintained proteins in the heart that were associated with a stress response[14]. The environment affected proteins, which later affected cell proliferation, etc. Overall, this research would support the bidirectional relationships between the processes underlying regeneration (i.e., DNA ↔ RNA ↔ protein ↔ structure ↔ function ↔ environmental experiences).

While there are several reviews on the role of the environment and experiences on regeneration[15–18], a common practice is to ignore or neglect their potential role. Probabilistic epigenesis—and the role of crowding on heart regeneration—suggests that the environment and social responses have an equal contribution to regeneration, such as gene expression, hormones, neural activity, etc. They are inseparable from one another. However, like many animal experiments[19], studies on regenerative animals neglect the role of the environment by housing them in simple and standardized environments to minimize potential interactions with the study question. From a DPB perspective, this minimization is virtually impossible because each animal is an individual with its own unique experiences or environments across development[20,21].

For example, individual differences cannot be “removed” from an inbred mouse housed in a standardized environment. These differences include sporadic gene changes via mini-satellite variation, the individual in utero experiences depending on the sex of their neighboring littermates, individual nutrition from their mother after birth, and different social dominance statuses in adulthood[22–26]. These unique experiences contribute to normal variability across a norm of reaction[27,28], and this norm of reaction is central to understanding development and regeneration. Only housing animals in a standardized environment limits our understanding of that reaction norm to regeneration in a simple environment. Whether our whole understanding of regeneration applies to any other environment remains unknown. Thus, manipulating the environments and experiences of regenerative animals would provide insight into the mechanisms underlying regeneration. Moreover, such research may help us fine-tune variables to improve regeneration outcomes in our research and clinics.

Another example of the importance of a probabilistic epigenesis perspective on regenerative biology concerns the role of biomechanical experiences on skeletal development. Classic studies in embryology paralyzed chick embryos during development and found improper joint development with bone fusions[29,30]. This paralysis was linked to differences in gene expression of mechanosensitive signaling pathways like *Wnt*, *Bmp*, and *Hippo*[31,32]. Thus, the movement of joints and limbs can affect bone development and likely regeneration.

This is further demonstrated by recent work in ecological developmental biology on the fin or “limb” development of *Polypterus* fish reared in aquatic or terrestrial environments[33,34]. *Polypterus* can survive on land as “tetrapods” or in water as fish, and they will move differently depending on the environment they are reared in. When reared on land, they intermittently pick up their nose and significantly twist their body back and forth while walking. However, when swimming, they keep their nose level, and their body will only slightly side to side. These differences in biomechanical forces (e.g., significant twisting vs. slight movements side to side) across development contribute to differences in the development of their skeletal system. For example, *Polypterus*, raised in a terrestrial environment, had more narrow and elongated bones in the fins and pectoral girdles. This illustrates the importance of mechanical loading and environmental experiences on bone development. Whether similar effects are present in bone and joint regeneration remains unclear, but imperfect skeletal morphologies occur in salamander limb regeneration, and the specific regulators remain unclear[35–38].

In summary, like development, the processes underlying regeneration coact with one another across regeneration in a form of probabilistic re-genesis. These processes traditionally include DNA, RNA, proteins, cells, the immune system, etc. However, they should also include behavior, environmental experience, and biomechanical processes and consider the inseparable nature of all underlying systems across regeneration (Fig. 1). Recent reviews on the environment and regeneration provide further examples[15–18]. However, these reviews often neglect these systems' coactional and inseparable nature.

“Development to” versus “Development from”

Now, I would like to provide more details on what development in DPB means and apply this perspective to regeneration. DPB commonly describes its perspective on development as “development from” rather than “development to”[39], or that development is “constructive” rather than “supportive”[40] (Fig. 2). This originates from

Lehrman[41,42] and Schneirla[43], who discuss that the “innate-acquired” (or nature-nurture) dichotomy restricts our understanding of the role development. From a “development to” perspective, genes or heredity specifies a predisposition for a phenotype, and the environment supports the manifestation of that phenotype as it unrolls. This allows experiences or environments to be permissive or non-permissive (i.e., disruptive) to phenotypic development. For example, you either develop the typical gait for walking or some aspect of the environment disrupts gait development to be abnormal or pathological. The same could be said for structural development; you either develop a perfectly formed limb or some aspect of the environment disrupted limb development to be abnormal or pathological[44]. This is because natural selection has selected for a specific heritable gene that encodes the adaptive trait (e.g., a “normal” gait or a body plan). However, the “development from” approach follows the probabilistic framework, applying equal weight to all underlying factors constructing the phenotype across development. In this case, natural selection has selected for the entire coactional system, which includes adaptability and an unknown range of phenotypes (e.g., a range of gaits or limb formations). Indeed, natural selection can only operate when variability exists. Thus, phenotypes “develop from” a coactional system constructing the phenotypic trait across development, and this developmental trajectory is unique to each individual rather than everyone “developing to” an encoded trait.

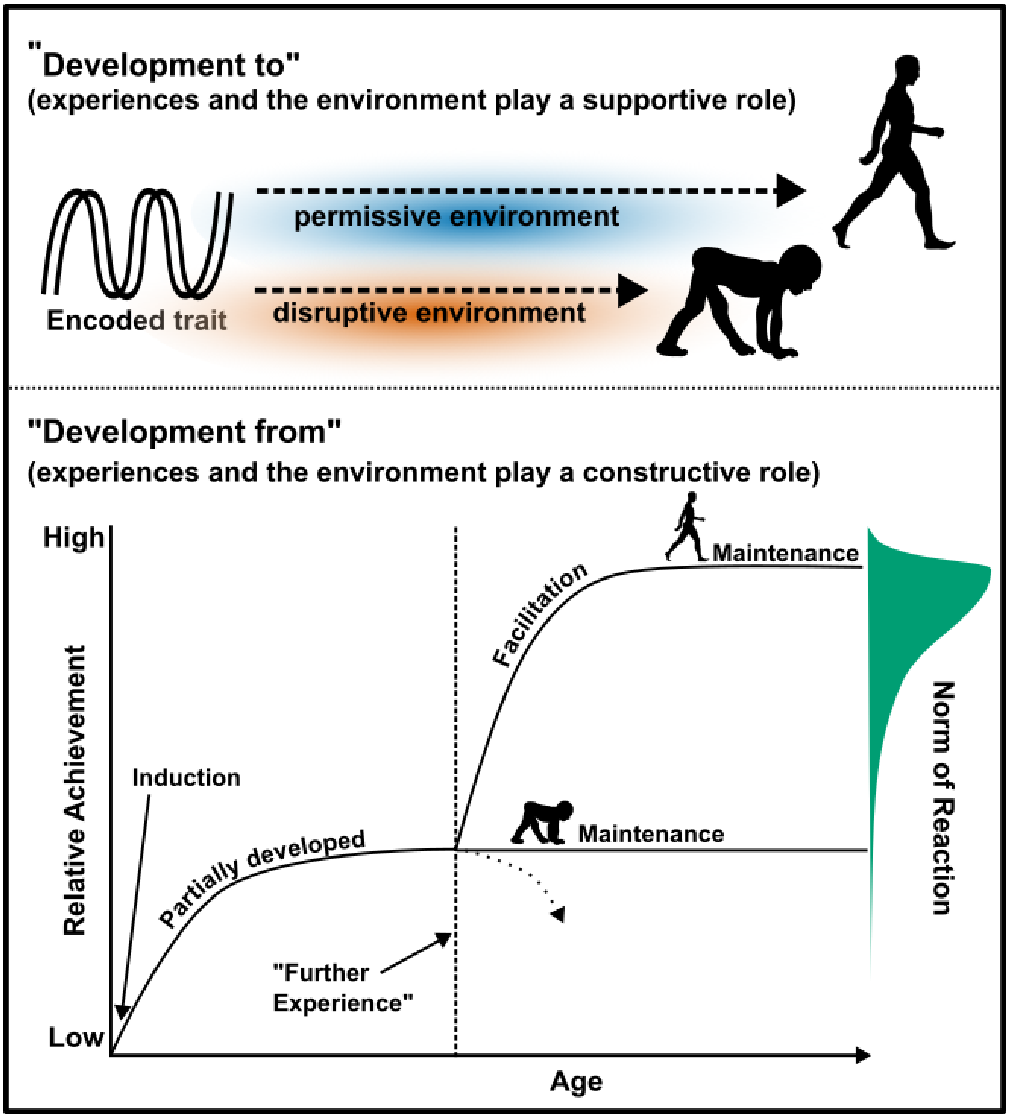

Fig 2. A comparison of "Development to" and "Development from". This is modified from Gottlieb's "Roles of Experience"(2) to include a diagram of "Development to" and the "Norm of Reaction." In the "Development to" section, an encoded trait symbolized by DNA unrolls in a permissive environment to allow for normative walking, or unrolls in a disruptive environment leading to pathological walking like "Uner Tan" Syndrome. In the "Development from" section, developmental trajectories are plotted across age on the x-axis depending on the relative high or low achievement on the y-axis. First, there is induction, perhaps leading to spontaneous movements, which then

increase in coordination over time. Further experience from birth allows for more sensory experiences to facilitate the development of walking. Or a specific behavior (e.g., crawling) never transforms and is maintained. These walking behaviors are neither normative nor pathological but occur on a norm of reaction, as shown by the imaginary distribution of walking behavior from the green bell curve on the far right.

While this perspective of “development from” is non-traditional, it reveals non-intuitive and counter-intuitive influences on the development of a phenotypic trait. Thus, it will likely reveal non-intuitive and counter-intuitive influences on regeneration and behavioral recovery. To employ a “development from” perspective, Gottlieb[5] and other DPB scientists[39] suggested that individual experiences contribute to three developmental consequences: (1) induction, where experience is necessary to alter the expression of a trait; (2) facilitation, where experience regulates the rate of development for the expression of a trait; and (3) maintenance, in which experience sustains a trait in an individual’s repertoire. While experience can be difficult to categorize within the reality of the coactional system, induction, facilitation, and maintenance can have significant consequences on development.

Studies on the development of walking illustrate the value of the “development from” perspective. For example, a “development to” perspective may assert that there is a set of genes for walking, demonstrated by the “primitive reflex” of stepping behavior at birth. The “primitive reflex” of stepping can be elicited in a newborn by supporting them under the arms and holding them upright, and they will make well-coordinated stepping movements. As the baby develops, this reflex disappears and then reappears as walking—as the encoded program unfolds[45]. Environmental factors can support or disrupt the unfolding of this encoded program.

In contrast, a "development from" perspective suggests there is no innate reflex; it is a manifestation of coordinating limb movements that continually transform due to sensorimotor experiences that normally occur and construct the phenotype across development. For example, walking in human infants can be induced to arrive earlier in development by daily facilitation with stepping practice, which also encourages longer durations of stepping before walking[46]. Thelen and colleagues also showed that the "primitive reflex" disappears because the limbs get heavier, causing stepping to manifest as kicking while lying on their back. Indeed, heavier babies have a later onset of walking than lighter babies[47]; ankle weights can decrease stepping, while submerging legs underwater increases stepping[48]; and a treadmill can help increase leg strength[49]. Thus, the "development from" perspective revealed the constructive and facilitative role of sensorimotor experiences on the transformations of air-stepping to kicking to crawling to walking.

The role of stepping experience can also be seen when comparing cultures. Jamaican parents expected their infants to walk at ten months, and most began walking independently at ten months. English parents expected their infants to walk at 12.5 months, while most began walking at 13 months[50]. Notably, a third of Jamaican infants skipped the crawling stage, compared to three out of 41 English infants. Also, several

Turkish families with “Uner Tan” syndrome walk on all fours. This abnormal style of walking is due to the maintenance of crawling behavior. A “development to” perspective, however, would assert that genetic mutations or factors of the environment did not support the normal unrolling of the encoded walking trait. They may also claim that quadrupedal walking is reminiscent of our evolutionary ancestors. However, careful behavioral analyses shows that crawling in “Uner Tan” syndrome is not similar to quadrupedal walking of our evolutionary ancestors[51]. Thus, the “development from” perspective revealed that different cultures create different environmental contexts for the development of walking behavior, altering the phases of facilitation and maintenance.

This “development from” perspective can be directly applied to tissue regeneration and behavioral recovery to understand the underlying processes better and inform potential therapies. Consider limb regeneration in axolotls and newts. The entire limb can be amputated and then allowed to regenerate. It is well established that some molecular pathways associated with limb development are used during limb regeneration[52]. This can lead one to ask: Are developmental pathways associated with walking also involved in behavioral recovery after injury? It is established in mice and rats that walking begins in utero with spontaneous limb movements that begin to have coordination due to sensorimotor feedback and biomechanical restrictions of the uterine environment[53,54]. So, when do the regenerating limbs begin showing spontaneous or coordinated movement during regeneration? When is the onset of coordinated stepping after injury? Can this onset be manipulated via practice stepping, etc.? How does a salamander behaviorally compensate for losing a limb and then continually recalibrate its nervous system as the limb regrows? Do biomechanical restrictions during regeneration affect the behavioral recovery of walking? What is the range of variability in the functional recovery of regenerated limbs? The answers to these questions remain unknown.

One attempt to modify—or facilitate—the outcome of regeneration with behavior comes from a study on hindlimb regeneration in newts. One hindlimb of the newt was amputated mid-femur, and they were forced to walk on a wet surface 48 hours after amputation, for five minutes, twice daily, five days a week for eight months[55]. A naïve developmental psychobiologist may predict that this locomotor training would facilitate the recovery of stepping behavior. However, the newts with locomotor training had delayed regeneration and heterogenous digit formation. Thus, the experience of stepping, which could theoretically increase stepping recovery, also led to poor structural regeneration. The authors note that the friction of the wound epidermis with the ground did not disrupt limb regeneration. This study suggests that newts naturally adopt a set of behaviors (e.g., compensatory, maintaining, inductive, facilitative, etc.) that aid in the synonymous regeneration and behavioral recovery of the limb. Importantly, this study neglected the neural underpinnings and recalibration for the loss and subsequent regeneration of the limb (i.e., neuroplasticity).

These “regeneration from” questions can be applied to any regenerative system that requires behavior to be functional: the spinal cord and locomotion (e.g., swimming and walking), the skin and sensation, muscle and movement, the optic nerve and vision, the axolotl brain and memory, the planaria pharynx and eating, breathing of the lungs, etc. Research investigating the typical arrival of behaviors after injury and methods to induce, transform, and maintain the behaviors are essential for translating regeneration and maximizing behavioral recovery after injury. Such research in humans has proved successful in spinal cord injury[56] and stroke[57]. This research should also exist on regenerative-competent systems and follow a “regeneration from” perspective.

Tinbergen’s Four Questions, plus one

When defining the aims and methods of ethology, Tinbergen developed four questions to serve as a roadmap for behavioral research[58]. The questions were derived from Thomas Huxley’s three major problems in biology: *function*, *proximate causation*, and *evolution*. Tinbergen added the fourth problem, or question, of *ontogeny*, and here I will add the fifth question of *regeneration* (Fig. 3). Each question should be examined separately as its own area of research. Answers to one question should not be answers to others. Once several questions are understood to an appreciable degree, they can be compared through discussion, and the answers can be integrated to understand the behavior further[1,58]. I will briefly expand on each question through examples of swimming behavior in fish since it is a common measure of behavioral recovery in regenerative biology[59–61].

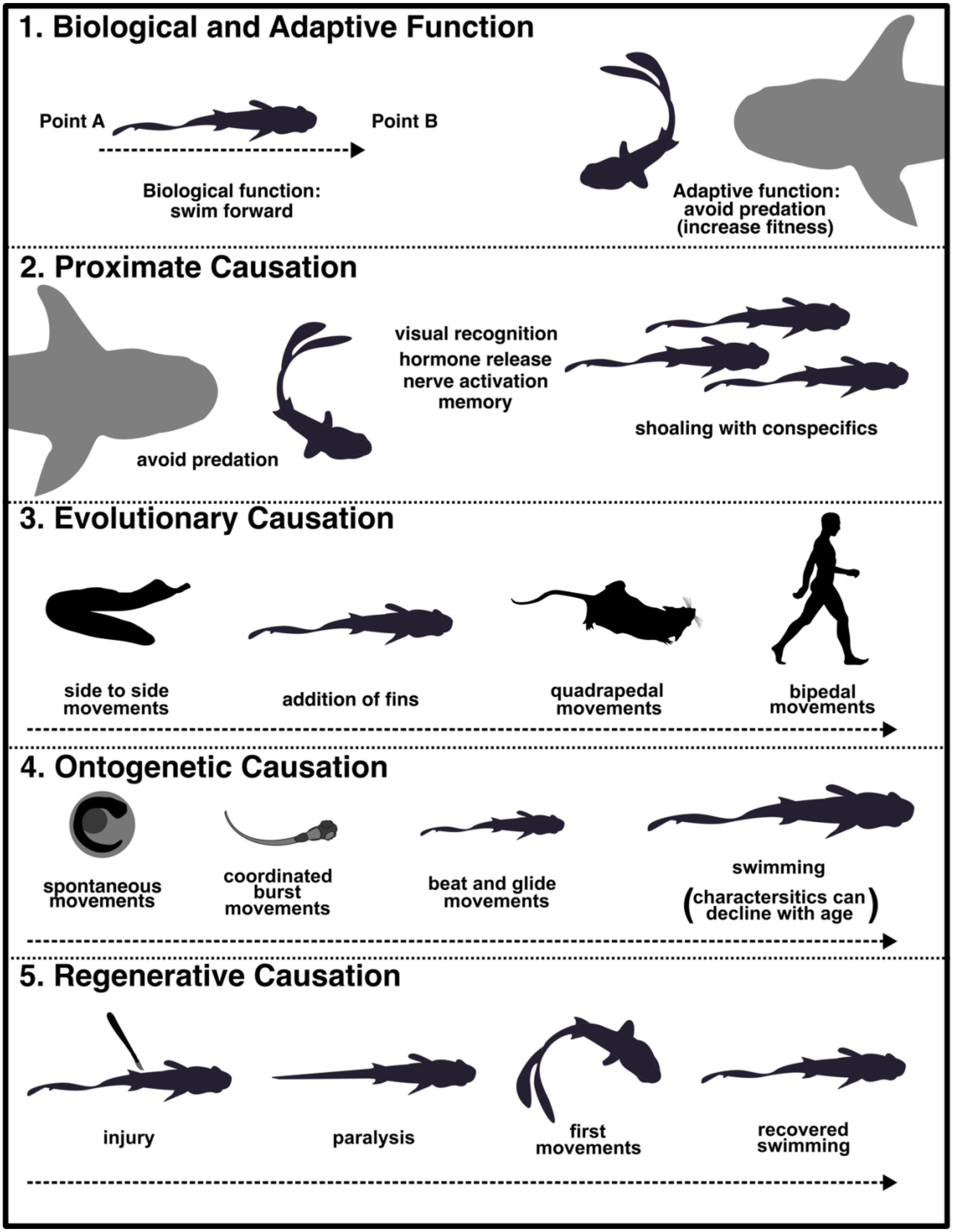
1. Biological and Adaptive Function
Point A
Point B
Biological function: swim forward
Adaptive function: avoid predation (increase fitness)
2. Proximate Causation
visual recognition
hormone release
nerve activation
memory
avoid predation
shoaling with conspecifics
3. Evolutionary Causation
side to side movements
addition of fins
quadrapedal movements
bipedal movements
4. Ontogenetic Causation
spontaneous movements
coordinated burst movements
beat and glide movements
swimming
(charactersitics can decline with age)
5. Regenerative Causation
injury
paralysis
first movements
recovered swimming

Fig 3: Summary diagram of Tinbergen's four questions plus regenerative causation. See text for details.

Biological and adaptive function question

Functional questions about behavior can be divided into biological and adaptive. For example, in the case of fish, swimming's biological function is to propel the fish forward in the water[62]. To determine swimming's adaptive function, however, one must test whether differences in swimming behaviors have a consequence for individual or group survival and reproduction (i.e., fitness).

Regenerative biologists attempting to understand the restoration of behavioral function after regeneration must recognize the biological and adaptive function of the behavior under investigation. Indeed, a fish might only recover their behavior if its biological and adaptive functions are fully restored. Determining the biological function of propelling forward is more or less straightforward because it does not matter *how* the regenerated fish swims forward, just that it does swim forward. Determining whether the adaptive function is restored, however, is more complex. One must understand what characteristics of swimming are adaptive. For instance, hydrodynamic thrust, fin kinematics, beats per second, peak beat velocity, etc. Each of these characteristics will have a phenotypic range for fitness. It is possible that the necessary evidence to determine the adaptive function of each characteristic in swimming is not yet understood. Until this is understood, regenerative biologists should at least compare the behaviors of regenerated and uninjured animals from the same developmental stage. Each individual's behaviors before injury could also be recorded, but changes in behavior could be confounded by the time it takes to regenerate the tissue. In summary, studying the biological function alone is insufficient for determining whether the behavioral function is restored.

Proximate causation question

The proximate causation question addresses the immediate causes leading to the behavior. This would relate to both external and internal influences. For instance, a fish might swim forward to catch prey, escape from a predator, or during shoaling behavior, etc. This swimming also relates to internal hormone levels, neural processes preceding the onset of swimming or its maintenance, memory, and gene activation. Proximate causation questions comprise the majority of research in physiology and psychology in relation to behaviors. To the regenerative biologist, the proximate causation of a behavior is critical to understanding whether similar proximate causations continue to initiate or maintain the behavior, etc.

Evolutionary history question

Evolution or phylogenetic history questions address the phylogenetic causes of behavior that can be traced back several generations to determine its precursors. The phylogenetic causation question can also be helpful in comparative research to understand which precursors animals share with humans, for example.

Phylogenetic research on swimming nicely demonstrates the power of such research. Swimming behavior has a long evolutionary history, and we can start with lancelets or amphioxus, typically considered the oldest common ancestor of vertebrates[63]. Lancelets swim using the coordination of their spinal cord and waves of movement in their epidermal cilia[64]. They also swim in chains, attaching themselves to others in a long chain and swimming in unison[65], and often swim backward to bury their tail in the sand[66]. As we progress through phylogenetic history, fish gain pectoral and dorsal fins and lose the epidermal cilia. Notably, cilia can be found in early development in some fishes, but that is related to ontogeny, which is a separate question[67]. Adding pectoral fins requires subsequent coordination of pectoral muscles in unison with side-to-side movement seen in lancelets. Also, with pectoral fins comes the addition of mechanosensation on the fins, which varies with fin morphology to assist with swimming and the eventual evolution of walking[68].

As previously mentioned, some fish like *Polypterus* can walk in terrestrial environments and swim in aquatic environments using their pectoral fins. Here, different sensorimotor feedback alters the skeletal system and the coordination and development of the spinal cord and central nervous system. As animals continue to walk, they evolve hindlimbs from the pelvic fins[69]. In the hindlimbs, we have the mechanosensory feedback and coordination with the spinal cord observed in the pectoral fins or forelimbs. Eventually, mechanosensory systems take on a primary role in the forelimbs with the evolution of bipedal walking. Thus, simple movements of the spinal cord in lancelets contributed to the evolution of swimming in fish, giving rise to mechanosensation in pectoral fins and the ability to walk on land, which later led to quadrupedal walking, and then bipedal walking—with an increase in forelimb mechanosensation.

This phylogenetic history is essential to the regenerative biologist, especially when studying behavior. Specific structures, like pectoral fins, may not regenerate, and the fish may adopt more lancelet-like swimming—albeit likely without chain swimming. Also, insight into the behavioral recovery of swimming may provide insight into walking since swimming is an evolutionary precursor to walking, and they share neural, muscular, and skeletal systems.

Ontogenetic history question

The fourth question is ontogenetic history, addressing the causes of a behavior across an animal's lifetime. One can track all the antecedent events in an individual’s life (i.e., the life history) related to the behavior to understand the ontogenetic precursors of a behavior and how the behavior has transformed across development. This question relates back to the “development from” versus “development to” discussion. Thus, depending on the perspective, a behavior might have different ontogenetic causes.

Like walking, spontaneous movements precede swimming behavior as a point of induction in swimming behavior. Spontaneous movements begin at 17 hours post fertilization (hpf), and their speed variably changes each hour[70]. At 21 hpf, the embryo responds to sensory stimulation, suggesting that the system is open to sensorimotor feedback changes around this time. From here, swimming movements become more frequent and powerful across development[70]. Upon hatching, 48 hpf, zebrafish larvae swim infrequently in undirected “bursts” lasting several seconds to a minute. By four days post fertilization (dpf), swimming follows a slower “beat-and-glide” pattern, where the beats are almost half as frequent as the “burst” pattern[71]. As zebrafish enter the juvenile and adult stage, their swimming diverges from the “beat-and-glide” pattern to something more infrequent and continuous, traversing longer distances with each swimming bout[72]. This variable pattern can be expected since adult fish more readily respond to environmental changes such as turbulence[73] and compensate for individual differences in body and fin size[74]. Some mutant zebrafish may also swim on their side, upside down, vertically, or have other anomalies[75,76]. As zebrafish get older, their ability to swim declines[77]. Understanding this ontogenetic process is important to understand the starting point of the behavior and locomotor system before the injury. It may also provide a model for the recovery of behavior, but again, that should be a separate question.

Regenerative history question

The final question concerns regenerative history, addressing all the behavioral and neural changes that occur after injury and until the system is maintained to a point of recovery. Since the ontogenetic history question covers the entire lifetime of the animal, regenerative history is just a special question or period that exists within the ontogenetic period. In most cases, the animal is paralyzed immediately after injury. This causes immediate and temporary changes to behavior and the neural underpinnings of the behavior as the animal compensates and recalibrates for the loss of function[78,79]. As axons regenerate into the regenerating tissue, behaviors return with spontaneous movement, transforming into coordination and behavioral recovery—as observed in spinal cord injury[80]. This pattern can be reminiscent of the original ontogenetic history of the behavior, similar to how regeneration follows some molecular signals associated with the molecular development of the regenerating structure. Notably, the regenerating adult's environment, the biomechanical interactions, and the time it takes to make the structure are markedly different from the embryo or neonate[81]. Thus, the morphological, biochemical, physiological, and behavioral mechanisms that occurred during development must be modified or suppressed during regeneration. This may result in a regeneration-specific adaptation that bears little to no resemblance to the original ontogenetic history. Once the regenerative history or regeneration-specific adaptation is understood, we may begin manipulating different aspects of the coactional system to modify the outcome and construction of regeneration and recovery.

To my knowledge, there don't appear to be any good examples of the regenerative history of a regenerating structure that consider this perspective at the moment. As an initial starting point, research from DPB would suggest that we should consider how the degree of structural regeneration, environmental constraints, and neuroplasticity coact to affect behavioral recovery across the time required to regenerate the tissue (Fig. 4). Any changes to these four factors (structure, environment, neuroplasticity, or time) will directly impact behavioral recovery. For example, delaying or increasing regeneration time will lead to differences in structural regeneration, altering environmental experiences and any changes in the brain (i.e., brain reorganization or neuroplasticity). It is important to first understand the "natural history" of regeneration from this perspective before empirically altering it since regenerative animals likely adopt novel behaviors or neuroplasticity to facilitate behavioral recovery.

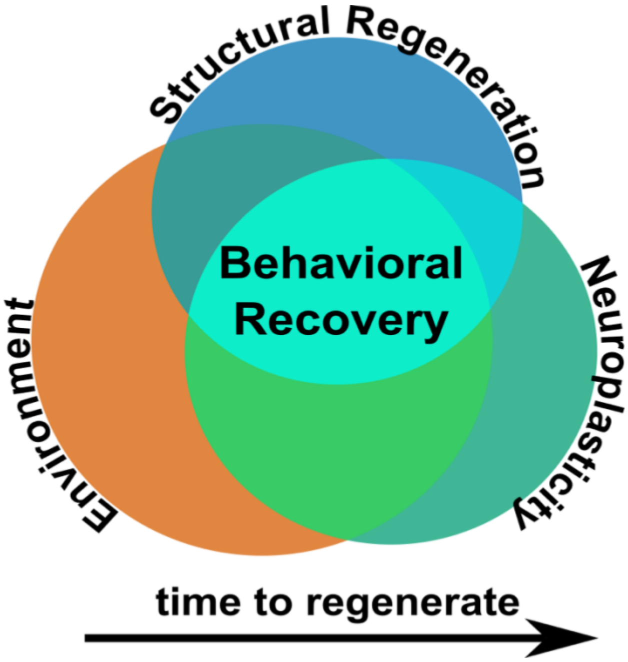

Fig 3: Initial framework for regenerative history research. Each circle represents either structural regeneration, the environment, or neuroplasticity. Each circle has a different area to illustrate different "degrees of x" (e.g., degrees of structural regeneration). The nexus of these circles is the degree of behavioral recovery. The time to regenerate is represented below the circles. As the animal regenerates, the area of the nexus, or intersection, will flux across regeneration time as the degree of each circle (i.e., the area) also changes. Understanding this triadic relationship across regenerative

time will be the firs step in understanding the regenerative history question in relation to behavioral recovery.

Conclusion

Integrating DPB into regenerative biology provides new research questions on behavioral recovery after injury and the role of behavior in regeneration. A probabilistic regenesis framework will allow behavior to contribute equally with molecular, cellular, and environmental components to the processes underlying regeneration. A “regeneration from” perspective will help identify factors that induce, facilitate, or maintain behavioral recovery. Tinbergen’s four questions and the question of regenerative causation will also help us better understand behavioral recovery regarding the restoration of function and proximate causes, how behavioral recovery relates to the evolution and ontogeny of the behavior, and how behaviors specific to regeneration may arise. Since DPB and regenerative biology are both sub-disciplines of developmental biology and include a focus on behavior, their integration would likely promote significant advancements in each.

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References

1. Michel, G. & Moore, C. *Developmental Psychobiology. An Interdisciplinary Science*. vol. 19 406–407 (1996).
2. Blass, E. M. *Handbook of Neurobiology*. vol. 13 (Springer, New York, NY, 2001).
3. Hall, W. G. & Oppenheim, R. W. Developmental psychobiology: prenatal, perinatal, and early postnatal aspects of behavioral development. *Annu. Rev. Psychol.* **38**, 91–128 (1987).
4. Amso, D. & Blumberg, M. S. 50th anniversary of developmental psychobiology. *Dev. Psychobiol.* **61**, 315–316 (2019).
5. Gottlieb, G. The roles of experience in the development of behavior and the nervous system. in *Studies on the Development of Behavior and the Nervous System* 25–54 (Elsevier, 1976).
6. Gottlieb, G. Normally Occurring Environmental and Behavioral Influences on Gene Activity: From Central Dogma to Probabilistic Epigenesis. *Psychol. Rev.* **105**, 792–802 (1998).
7. Weiss, P. Cellular Dynamics. *Rev. Mod. Phys.* **31**, 11–20 (1959).
8. Wright, S. *Evolution and the Genetics of Populations: Variability Within and Among Natural Populations*. (1968).
9. Gottlieb, G. *Individual Development and Evolution: The Genesis of Novel Behavior*. (Oxford University Press, 1992).
10. Crain, D. A. & Guillette, L. J., Jr. Reptiles as models of contaminant-induced endocrine disruption. *Anim. Reprod. Sci.* **53**, 77–86 (1998).
11. Kirschner, M., Gerhart, J. C., Hara, K. & Ubbels, G. A. Initiation of the Cell Cycle and Establishment of Bilateral Symmetry in Xenopus Eggs. in *The Cell Surface: Mediator of Developmental Processes* (ed. Subtelny, S.) 187–215 (Academic Press, 1980).
12. Shields, J. *Monozygotic Twins Brought Up Apart and Brought Up Together: An Investigation Into the Genetic and Environmental Causes of Variation in Personality*.

(Oxford University Press, 1962).

13. Sallin, P. & Jaźwińska, A. Acute stress is detrimental to heart regeneration in zebrafish. *Open Biol.* **6**, 160012- (2016).
14. Geary, B. *et al.* Acute stress alters the rates of degradation of cardiac muscle proteins. *J. Proteomics* **191**, 124–130 (2019).
15. Easterling, M. R., Engbrecht, K. & Crespi, E. J. Endocrine regulation of epimorphic regeneration. *Endocrinology* **160**, 2969–2980 (2019).
16. Easterling, M. R., Engbrecht, K. M. & Crespi, E. J. Endocrine regulation of regeneration: Linking global signals to local processes. *Gen. Comp. Endocrinol.* **283**, 113220 (2019).
17. Losner, J., Courtemanche, K. & Whited, J. L. A cross-species analysis of systemic mediators of repair and complex tissue regeneration. *npj Regenerative Medicine* **6**, 1–11 (2021).
18. Tung, A. & Levin, M. Extra-genomic instructive influences in morphogenesis: A review of external signals that regulate growth and form. *Dev. Biol.* **461**, 1–12 (2020).
19. van Zutphen, L. F. M., Baumans, V. & Beynen, A. C. *Principles of Laboratory Animal Science : A Contribution to the Humane Use and Care of Animals and to the Quality of Experimental Results*. 389 (Elsevier, 1993).
20. Bateson, P. The return of the whole organism. *J. Biosci.* **30**, 31–39 (2005).
21. Wahlsten, D. Behavioral Genetics. in *Encyclopedia of Psychology* (ed. Kazdin, A. E.) 378–385 (American Psychological Association and Oxford University Press Inc., New York, 2000).
22. Lathe, R. The individuality of mice. *Genes Brain Behav.* **3**, 317–327 (2004).
23. Ryan, B. C. & Vandenbergh, J. G. Intrauterine position effects. *Neurosci. Biobehav. Rev.* **26**, 665–678 (2002).
24. Dumont, B. L. Significant strain variation in the mutation spectra of inbred laboratory mice. *Mol. Biol. Evol.* **36**, 865–874 (2019).

25. Barnard, C. J., Behnke, J. M., Gage, A. R., Brown, H. & Smithurst, P. R. Maternal effects on the development of social rank and immunity trade-offs in male laboratory mice (Mus musculus). *Proc. Biol. Sci.* **265**, 2087–2093 (1998).
26. Varholick, J. A. *et al.* Social dominance hierarchy type and rank contribute to phenotypic variation within cages of laboratory mice. *Sci. Rep.* **9**, 1–11 (2019).
27. Woltereck, R. Weitere experimentelle Untersuchungen über Artveränderung, speziel über das Wesen quantitativer Artunterschiede bei Daphniden. *Verh. Dtsch. Zool. Ges.* **1909**, 110–172 (1909).
28. Platt, S. A. & Sanislow, C. A., 3rd. Norm-of-reaction: definition and misinterpretation of animal research. *J. Comp. Psychol.* **102**, 254–261 (1988).
29. Murray, P. D. F. & Drachman, D. B. The role of movement in the development of joints and related structures: the head and neck in the chick embryo. *Development* **22**, 349–371 (1969).
30. Hogg, D. A. & Hosseini, A. The effects of paralysis on skeletal development in the chick embryo. *Comp. Biochem. Physiol. A Physiol.* **103**, 25–28 (1992).
31. Rolfe, R. A. *et al.* Identification of mechanosensitive genes during skeletal development: alteration of genes associated with cytoskeletal rearrangement and cell signalling pathways. *BMC Genomics* **15**, 48 (2014).
32. Rolfe, R. A., Shea, C. A., Singh, P. N. P., Bandyopadhyay, A. & Murphy, P. Investigating the mechanistic basis of biomechanical input controlling skeletal development: exploring the interplay with Wnt signalling at the joint. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **373**, 20170329 (2018).
33. Standen, E. M., Du, T. Y. & Larsson, H. C. E. Developmental plasticity and the origin of tetrapods. *Nature* **513**, 54–58 (2014).
34. Du, T. Y. & Standen, E. M. Terrestrial acclimation and exercise lead to bone functional response in Polypterus senegalus pectoral fins. *J. Exp. Biol.* **223**, (2020).

35. Young, H. Anomalies of Limb Regeneration in the Adult Salamander, Ambystoma annulatum. *Journal of the Arkansas Academy of Science* **31**, 110–111 (1977).
36. Gassner, K. M. & Tassava, R. A. Abnormal limb regeneration in the short-toes mutant of the axolotl, Ambystoma mexicanum: studies of age, level of amputation, and extracellular matrix. *J. Exp. Zool.* **279**, 571–578 (1997).
37. Monaghan, J. R. *et al.* Experimentally induced metamorphosis in axolotls reduces regenerative rate and fidelity. *Regeneration (Oxford, England)* **1**, 2–14 (2014).
38. Bothe, V., Mahlow, K. & Fröbisch, N. B. A histological study of normal and pathological limb regeneration in the Mexican axolotl Ambystoma mexicanum. *J. Exp. Zool. B Mol. Dev. Evol.* **336**, 116–128 (2021).
39. Michel, G. F. & Tyler, A. N. Developing human nature: “Development to” versus “Development from?” *Dev. Psychobiol.* **49**, 788–799 (2007).
40. Kohn, G. M. Revisiting T. c. schneirla’s “interrelationships of the ‘innate’ and the ‘acquired’ in instinctive behavior” (1956). *Biol. Theory* **19**, 84–93 (2024).
41. Lehrman, D. S. Semantic and Conceptual Issues in the Nature-Nurture Problem. in *Development and evolution of behavior* (eds. Aronson, L. R., Tobach, E., Lerhman, D. S. & Rosenblatt, J. S.) (Freeman, San Francisco, 1970).
42. Lehrman, D. S. A Critique of Konrad Lorenz’s Theory of Instinctive Behavior. *Q. Rev. Biol.* **28**, 337–363 (1953).
43. Schnierla, T. C. The interrelationships of the “innate” and the “acquired” in instinctive behavior. in *L’Instinct dans le comportement des animaux et de l’homme* (ed. Grassé, P. P.) 78–108 (Masson, Paris, 1957).
44. Alexander, P. G., Clark, K. L. & Tuan, R. S. Prenatal exposure to environmental factors and congenital limb defects: ENVIRONMENTAL FACTORS IN CONGENITAL LIMB DEFECTS. *Birth Defects Res. C Embryo Today* **108**, 243–273 (2016).
45. McGraw, M. B. Neuromuscular development of the human infant as exemplified in the

achievement of erect locomotion. *J. Pediatr.* **17**, 747–771 (1940).

46. Zelazo, P. R., Zelazo, N. A. & Kolb, S. “Walking” in the newborn. *Science* **176**, 314–315 (1972).

47. Thelen, E., Fisher, D. M., Ridley-Johnson, R. & Griffin, N. J. Effects of body build and arousal on newborn infant stepping. *Dev. Psychobiol.* **15**, 447–453 (1982).

48. Thelen, E., Fisher, D. M. & Ridley-Johnson, R. The relationship between physical growth and a newborn reflex. *Infant Behav. Dev.* **7**, 479–493 (1984).

49. Thelen, E. Treadmill-elicited stepping in seven-month-old infants. *Child Dev.* **57**, 1498 (1986).

50. Hopkins, B. & Westra, T. Maternal expectations of their infants’ development: some cultural differences. *Dev. Med. Child Neurol.* **31**, 384–390 (1989).

51. Shapiro, L. J. *et al.* Human quadrupeds, primate quadrupedalism, and Uner Tan Syndrome. *PLoS One* **9**, e101758 (2014).

52. Zhong, J. *et al.* Multi-species atlas resolves an axolotl limb development and regeneration paradox. *Nat. Commun.* **14**, 6346 (2023).

53. Robinson, S. R. & Smotherman, W. P. Environmental determinants of behaviour in the rat fetus. II. The emergence of synchronous movement. *Anim. Behav.* **35**, 1652–1662 (1987).

54. Robinson, S. R. & Smotherman, W. P. The amniotic sac as scaffolding: prenatal ontogeny of an action pattern. *Dev. Psychobiol.* **24**, 463–485 (1991).

55. Launay, T., Cabelguen, J. M., Marini, J. F. & Chanoine, C. Effects of locomotor training on hindlimb regeneration in the urodele amphibian Pleurodeles waltlii. *J. Physiol.* **535**, 241–248 (2001).

56. Hornby, T. G., Zemon, D. H. & Campbell, D. Robotic-Assisted, Body-Weight–Supported Treadmill Training in Individuals Following Motor Incomplete Spinal Cord Injury. *Phys. Ther.* **85**, 52–66 (2005).

57. Taub, E., Crago, J. E. & Uswatte, G. Constraint-induced movement therapy: A new

approach to treatment in physical rehabilitation. *Rehabil. Psychol.* **43**, 152–170 (1998).

58. Tinbergen, N. On aims and methods of Ethology. *Z. Tierpsychol.* **20**, 410–433 (2010).
59. Burris, B., Jensen, N. & Mokalled, M. H. Assessment of Swim Endurance and Swim Behavior in Adult Zebrafish. *J. Vis. Exp.* (2021) doi:10.3791/63240.
60. van Raamsdonk, W., Maslam, S., de Jong, D. H., Smit-Onel, M. J. & Velzing, E. Long term effects of spinal cord transection in zebrafish: swimming performances, and metabolic properties of the neuromuscular system. *Acta Histochem.* **100**, 117–131 (1998).
61. Rasmussen, J. P. & Sagasti, A. Learning to swim, again: Axon regeneration in fish. *Exp. Neurol.* **287**, 318–330 (2017).
62. Richards, C. T. Kinematics and hydrodynamics analysis of swimming anurans reveals striking inter-specific differences in the mechanism for producing thrust. *J. Exp. Biol.* **213**, 621–634 (2010).
63. D'Aniello, S., Bertrand, S. & Escriva, H. Amphioxus as a model to study the evolution of development in chordates. *Elife* **12**, (2023).
64. Stokes, M. D. Ontogenetic changes in the morphology, ecology and locomotory biomechanics of the lancelet, Branchiostoma floridae. (University of California, San Diego, 1996).
65. Gudger, E. W. Amphioxi Swimming in a Chain. *Copeia* **1945**, 113 (1945).
66. Arey, L. B. The orientation of amphioxus during locomotion. *J. Exp. Zool.* **19**, 37–44 (1915).
67. Kemp, A. Role of epidermal cilia in development of the Australian lungfish, Neoceratodus forsteri (Osteichthyes: Dipnoi). *J. Morphol.* **228**, 203–221 (1996).
68. Aiello, B. R., Westneat, M. W. & Hale, M. E. Mechanosensation is evolutionarily tuned to locomotor mechanics. *Proc. Natl. Acad. Sci. U. S. A.* **114**, 4459–4464 (2017).
69. Don, E. K., Currie, P. D. & Cole, N. J. The evolutionary history of the development of the pelvic fin/hindlimb. *J. Anat.* **222**, 114–133 (2013).
70. Saint-Amant, L. & Drapeau, P. Time course of the development of motor behaviors in the

zebrafish embryo. *J. Neurobiol.* **37**, 622–632 (1998).

71. Buss, R. R. & Drapeau, P. Synaptic drive to motoneurons during fictive swimming in the developing zebrafish. *J. Neurophysiol.* **86**, 197–210 (2001).
72. Fuiman, L. A. & Webb, P. W. Ontogeny of routine swimming activity and performance in zebra danios (Teleostei: Cyprinidae). *Anim. Behav.* **36**, 250–261 (1988).
73. Liao, J. C. A review of fish swimming mechanics and behaviour in altered flows. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **362**, 1973–1993 (2007).
74. Plaut, I. Effects of fin size on swimming performance, swimming behaviour and routine activity of zebrafish Danio rerio. *J. Exp. Biol.* **203**, 813–820 (2000).
75. Drapeau, P. *et al.* Development of the locomotor network in zebrafish. *Prog. Neurobiol.* **68**, 85–111 (2002).
76. Kalueff, A. V. *et al.* Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish* **10**, 70–86 (2013).
77. Gilbert, M. J. H., Zerulla, T. C. & Tierney, K. B. Zebrafish (Danio rerio) as a model for the study of aging and exercise: physical ability and trainability decrease with age. *Exp. Gerontol.* **50**, 106–113 (2014).
78. Lundborg, G. Losing a Hand. in *The hand and the brain* 155–164 (Springer London, London, 2014).
79. Cromie, G. L. & Chapple, D. G. Impact of tail loss on the behaviour and locomotor performance of two sympatric Lampropholis skink species. *PLoS One* **7**, e34732 (2012).
80. Sławińska, U., Majczyński, H. & Djavadian, R. Recovery of hindlimb motor functions after spinal cord transection is enhanced by grafts of the embryonic raphe nuclei. *Exp. Brain Res.* **132**, 27–38 (2000).
81. Oppenheim, R. W. Ontogenetic adaptations and retrogressive processes in the development of the nervous system and behavior: A neuroembryological perspective. *Maturation and development: Biological and psychological perspectives* 73–109 (1981).