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► To cite this version:

Chiara Sinigaglia, Alexandre Alié, Stefano Tiozzo. The Hazards of Regeneration: From Morgan's Legacy to Evo-Devo. *Whole-Body Regeneration*, 2450, Springer US, pp.3-25, 2022, *Methods in Molecular Biology*, 10.1007/978-1-0716-2172-1_1 . hal-03815577

HAL Id: hal-03815577

<https://cnrs.hal.science/hal-03815577v1>

Submitted on 24 Nov 2022

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The hazards of regeneration: from Morgan's legacy to evo-devo

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Running title: From Morgan's legacy to evo-devo

Keywords: whole-body regeneration, evolution, phylogeny, adaptation, homology, character, ecology, development

Abstract

In his prominent book, *Regeneration* (1901), T.H. Morgan's collected and synthesized theoretical and experimental findings from a diverse array of regenerating animals and plants. Through his endeavor, he introduced a new way to study regeneration and its evolution, setting a conceptual framework that still guides today's research and that embraces the contemporary evolutionary and developmental approaches.

In the first part of the paper, we summarize Morgan's major tenets and use it as a narrative thread to advocate interpreting regenerative biology through the theoretical tools provided by evolution and developmental biology, but also to highlight potential caveats resulting from the rapid proliferation of comparative studies and from the expansion of experimental laboratory models. In the second part, we review some experimental evo-devo approaches, highlighting their power and some of their interpretative dangers. Finally, in order to further understand the evolution of regenerative abilities, we portray an adaptive perspective on the evolution of regeneration and suggest a framework for investigating the adaptive nature of regeneration.

1. Introduction

Thomas Hunt Morgan is considered one of the fathers of modern genetics. He is best known for demonstrating that chromosomes carry the mechanical basis of heredity, the genes. He also has the merit of introducing and developing a successful laboratory model for genetic studies, the fruit fly *Drosophila*. Yet, in his early career, while working at the Bryn Mawr women's college (1891-1904), Morgan devoted a significant amount of time to studying the problem of regeneration, focusing on a diverse array of regenerating animals (Fig. 1). Morgan's experimental and theoretical findings are synthesized in his now-classic book *Regeneration*[1]. Despite his extensive experiments and the diversity of the organisms studied, Morgan failed to identify a universal mechanism governing regeneration. Probably in a lighter moment, he allegedly said that since he had been unable to solve the problem of regeneration, he had decided to try something easier such as the problem of heredity[2]. The fascination and the struggle of understanding regenerative phenomena and their evolution remain as alive today as it was then.

Over the last two decades, new cell and molecular biology tools have become available, allowing the exploration of a broader range of metazoan regenerative mechanisms and prompting a (re)expansion of the field of regenerative biology[3, 4]. A unifying theory of regeneration is nevertheless still lacking. Why do not all species regenerate? Does regeneration have a single or multiple (evolutionary) origin? Are the mechanisms of regeneration co-opted from other developmental phenomena (i.e. embryogenesis)? To what extent asexual reproduction, coloniality, cancer, and regeneration can be seen as different facets of the same phenomenon? Can we decipher the mechanisms of

regeneration and re-enable them in non-regenerating species? Such compelling questions are still waiting for satisfactory answers.

Morgan's book[1] is as relevant today as it was in the previous century, as, besides providing a historical perspective on regeneration studies across the 19th and the 20th century, it lays down the conceptual and theoretical framework guiding our current research on regenerative phenomena.

2. The legacy of Morgan's *Regeneration*

In *Regeneration*, Morgan synthesized and critically revised the work of his colleagues and predecessors. By analyzing classical studies, including the work of Trembley, Spallanzani and Bonnet, and the ongoing work of his contemporary scholars, such as Roux, Barfurth and Driesch, Morgan realized how the results diverged significantly in relation to the organism studied and the methodology adopted, often leading to controversial interpretations. Through his exercise of synthesis, Morgan first attempted to group organism-specific processes into a general phenomenon of regeneration, framing his comparative approach into general questions concerning growth and differentiation, and eventually providing new insights to a theory of development. Indeed, one of the most important contributions of Morgan's book was the idea that regeneration should be considered as a growth property, and therefore approached as a developmental phenomenon. This approach to regeneration actively opposed the adaptationist view endorsed by August Weismann[5, 6], who considered regeneration as a phenomenon of adaptation and not a primary quality of the organism[7], and supported the existence of a causal relationship between the tendency to be injured and the capacity to "*re-grow*". With

the filter of time, the inflamed debate between the two scientists was most likely rooted on methodological and epistemological grounds, with Morgan criticizing Weismann for his adherence to a "theory", instead of starting from a purely experimental approach[5]. These originally discordant approaches are not mutually exclusive, and studying regeneration today as a form of development does not mean that this process has to be considered irrespectively of its adaptive value[8].

Morgan advocated and emphasized the importance of comparing the widest diversity of organisms in order to recast the questions about development in terms of experimentally testable hypotheses. His view of regeneration was supported by a striking array of experiments that he and his students performed on a substantial number of vertebrate and invertebrate species (Fig. 1). Undeniably, the tenet that emerges in *Regeneration* and that is still acutely pertinent 120 years later is to challenge any general hypothesis about regenerative phenomena by performing comparative experiments using different model organisms[1, 6, 9].

2.1 Partial versus Whole-Body Regeneration

In the pursuit of a coherent explanation of regenerative phenomena, one of the priorities in Morgan's work was to introduce a clearer and more consistent terminology, able to reflect the variety of regenerative processes and to compare the many models that he and his students were describing. Even if Morgan's most famous dichotomous subdivision of regeneration based on cellular rearrangements (morphallaxis) and cell proliferation (epimorphosis) turned out to be too restrictive[10], some of his terminology and classifications are still relevant today. For instance, Morgan classified regenerative

ontogenies according to the new anatomical structures that resulted from regeneration [1]. Another general classification provided by Morgan is based on the causality of the regenerative process. He distinguished between “*restorative regeneration*”, which include post-traumatic regeneration and is the result of some exogenous injury to the organism, and “*physiological regeneration*”, which occurs during body homeostasis, such as the turnover cycle of epithelial dermal cells in mammals, or during the “life-cycle of the individual”, like for example during budding, molting or feather replacements.

To our knowledge, the expression *whole-body regeneration* (WBR) was not used in Morgan’s work. It has been introduced relatively recently and spread widely in the scientific literature [11–18]. The term WBR has been loosely used to describe regenerative processes that involve a “large” portion of an animal body, without adhering to a strict definition. According to Cary and colleagues, an organism undergoes WBR when it “[...] *can re-grow all body parts following amputation*”, which is opposed to “*partial regeneration*”, when regeneration is restricted to only some body structure [16]. Bely and colleagues also define WBR as the ability to regenerate “*all body parts*”, and considered that regeneration of the primary body axis is not by itself sufficient to define WBR[4]. When using WBR most authors refer to restorative regeneration but it has also been used for physiological regenerative processes[19, 20]. The expression is also employed regardless of the stages of an organism's life cycle [16, 17].

While venturing into a clearer definition of WBR we run into some classical philosophical problems. WBR brings to the forefront the problem of biological individuality and, more specifically, the issue of establishing criteria for the persistence over time of biological individuals[21, 22]: to which and how many changes an organism can go through and still

be considered the same individual? When WBR leads to two or more individuals how regeneration can be considered different from reproduction, and which one is the original individual? Indeed, the expression “WBR” is rather idiomatic since, if an injury leaves some cells or tissues behind, the regeneration then cannot be “whole”. It appears that the amount of regenerated material is the main property defining WBR, but what is the threshold above which regeneration can be labeled “whole”? We could consider, for example, that at least half of the original individual has to regenerate. Following this rule, in a beheaded *Planaria maculata* the head reforming the body would be a case of WBR, but not the body reforming the head[23] (Fig. 1B). Yet, such a threshold would be clearly arbitrary, leading to conclusions that would need to be justified.

The term “whole-body regeneration” has become popular only in the last few decades. Just like the use of “regeneration”, it is rich in emphasis, but not accurate and nor fully definable. Regardless of the criteria to define it, WBR in different species clearly refers to different processes.

2.2 Regeneration: function versus process

While attempting to introduce a language that accommodates the various regenerative phenomena that had been studied so far, Morgan used the term *regeneration* to indicate diverse and heterogeneous phenomena of organ renewal, replacements of body parts, or asexual development[6]. He wrote that “regeneration” could constitute an umbrella term encompassing “*not only the replacement of a lost part, but also the development of a new, whole organism, or even a part of an organism, from a piece of an adult, or of an embryo, or of an egg*”, and even including instances of imperfect regeneration: “[...] *must*

include also those cases in which the part replaced is less than the part removed, or even different in kind”[1]. This broad definition of regenerative phenomena is still applied today. Just like WBR, it should however be regarded as a “working definition”, encompassing a heterogeneous class of events, not necessarily shared among taxa[24, 25]. Despite the complexity of the phenomena considered and the blurriness of definitions, often there has been a tendency to map regeneration as a character on phylogenetic trees. However, regeneration cannot be reduced to a single trait, and plotting onto an existing phylogeny its presence or absence has no more value than charting the capacity of animals to fly instead of focusing on the mechanisms and structures that allow the flight. Indeed, functions can arise convergently by multiple means rather than by historical continuity[26]. **Instead, regeneration must be considered as a spatio-temporal organized process, or assemblies of processes into modules [27, 28] that can be used** as individual evolutionary characters [29, 30]. Then, only characters on which we can do a reasonable hypothesis of primary homology[31], for example morphological, cellular, or molecular characters associated with regeneration, can be plotted on a tree.

To identify characters associated with regeneration it may be convenient to move towards a more reductionist approach, and break down the regenerative process along its ontogenetic and evolutionary paths. In the first case, each regenerative process could be split into conserved sub-processes such as wound-healing (when present), precursor(s) mobilization, and morphogenesis[32]. The latter involves comparing these artificial ontogenetic steps between closely related phylogenetic clades, e.g. class, order or family, minimizing divergence time[25, 33, 34]. The definition and the breakdown of components,

and the identification of which, if any, descend from a common ancestor are among the key interests of the field of evo-devo.

2.3 Help from evo-devo theoretical tools

If, as Morgan firstly suggested, regenerative phenomena can be considered as developmental processes, then the conceptual and methodological approaches developed by evo-devo research are valuable also to explore the evolution of regenerative processes[3, 19, 25, 35]. First, the use of an extended concept of homology, such as “process homology” [29] or “character identity networks”[26], which links characters from different biological hierarchies (e.g. gene, GRN, morphological characters), and, for instance, can help to describe relationships between homologous proteins and homologous molecular pathways, **even if they** do not necessarily lead to homologous anatomical structures[29, 36]. This more nuanced concept of homology is a powerful tool to refine comparisons of apparently unrelated regenerative processes, potentially also among phylogenetically distant and divergent species.

Second, another useful concept that captures the different levels and types of heterogeneity of an organism is the notion of *modularity*[27, 37, 38]. Regeneration, just like development can be divided into discrete and interacting modules, which can be tissues, fields (i.e. cells committed to forming the same structure), elements of gene enhancers, parts of gene regulatory networks, or any other “*basic structural entities or regulatory phenomena necessary to assemble a complex morphological structure*”[39]. The concept of modules also helps to distinguish the processes occurring during regeneration from the function of regeneration itself [25].

Third, conjointly with modularity comes the concept of *developmental constraint*, which restrains phenotype production due to a limited interaction among existing modules[29, 40]. For example, a limited or restrained propagation of morphogens, or bioelectric signals through voltage gradients, due to the increased histological and cytological complexity could prevent regeneration[41, 42]. The possible inhibitory effect of the immune system on regeneration is also another little-studied potential constraint [43–45]. The existence of developmental constraints should also be taken into account when comparing regenerative processes across different species.

The conceptual tools that regenerative biology can borrow from the field of evo-devo are powerful. Comparative approaches however entail interpretive caveats, as illustrated in the following examples.

3 The difficult task of reconstructing WBR evolution

The evolutionary questions concerning regeneration ultimately provide a complete narrative of the phenomenon. They are far from being just theoretical, and they can change the approach to the mechanistic study and guide the experimental design on a given model organism[8]. The three following examples illustrate the power of evo-devo experimental approaches to infer the evolution of regeneration - and of WBR in particular - but also point out some possible interpretive caveats.

3.1. Far from basal: diversity of regeneration in sponges

Sponges are emblematic organisms to study the early evolution of regeneration because they have excellent regenerative abilities[46] and likely represent the monophyletic sister

group of all other metazoans[47, 48]. Sponges are often considered as basal metazoans, or ancestral representative of animals. However, they are not more basal to eumetazoans than eumetazoans are basal to sponges (Fig. 2A), and there is no fossil evidence that their body plan represents an ancient state[49, 50]. As any organism, modern sponges are nothing but a mosaic of characters in their ancestral or derived state. This holds true for their regenerative mechanisms that show great inter-species variations. For instance, the proverbial ability of cell aggregates to generate a functional sponge varies even between closely related species[51–54]: *Halisarca dujardini* can reconstruct its body from cell suspension, whereas *Halisarca panicea* is unable to do so [53]. Whether cell re-aggregation is ancestral to Porifera will remain unsolved without phylum-level comparative studies.

The mechanisms of WBR from body fragment also varies between the four sponges classes. Many demosponges use massive proliferation and migration of archaeocytes with the participation of dedifferentiated choanocytes, which all together form a regenerative blastema[55, 56]. In some other demosponges (e.g. *Halisarca dujardini* and *Aplysina cavernicola*) the cell plasticity is even greater, with dedifferentiation of various cell types that also participate in blastema formation[57, 58]. In contrast to demosponges, neither archaeocytes nor tissue regeneration have yet been observed in their sister group, the Hexactinellida[59]. Calcareous sponges, who also do not possess archaeocytes, regenerate through epithelial morphogenesis by spreading and transdifferentiation of pinacocytes and choanocytes (e.g. in *Leucosolenia complicata*) with minor cell proliferation and no blastema formation[60]. Finally, among the homoscleromorphs, the sister group of calcareous sponges, only *Oscarella lobularis* has been reported to

regenerate[61, 62]. As in Calcarea, it involves choanocyte transdifferentiation and tissue rearrangement, without blastema formation or local proliferation. Due to this phylum-level variability in regenerative capability and mechanisms, reconstructing the origin and evolution of WBR in sponges is far from being a straightforward task (Fig. 2A).

Nevertheless, choanocyte dedifferentiation and/or transdifferentiation seem to be a common theme in regenerative species, which may be in line with the suspected stem cell nature of choanocytes[63]. Comparative investigations focused on choanocyte dynamics (e.g. time series of single-cell RNAseq) could unravel fundamental sets of genes regulating WBR potentially inherited from the last common ancestor of Porifera.

3.2. Acoels and planarians: lessons from faraway cousins

Recent work on acoels and Platyhelminthes has provided fresh insights on the possible ancestral mechanisms of WBR in the last common ancestor of Bilateria. Acoels are flatworms belonging to a larger clade named Xenacoelomorpha, together with Xenoturbellids and Nemertodermatids (Fig. 2B). Some authors consider Xenacoelomorpha as the sister group of all other Bilateria[64, 65] and others the sister group of Ambulacraria. Despite being distantly related, acoels share a superficial morphological resemblance with Platyhelminthes, a group of lophotrochozoan flatworms. Their regenerative mechanisms also show extensive similarities. In acoels and planarians, regeneration involves the proliferation-dependent formation of a regenerative blastema by mesenchymal multipotent and totipotent stem cells, the neoblasts, which express homologous genes such as *Piwi* paralogues and other members of the Germline Multipotency Programs[66–68]. In both acoels and planarians, muscles play a

contraction-independent role by secreting position control proteins (e.g. wnt and bmp ligands), thus providing positional information for correct body plan restoration upon WBR[69–72]. These shared characters suggest ancestral features inherited from the last common ancestor of Bilateria. However, proposing the homology of regenerative processes at such a large phylogenetic scale remains risky. For instance, while neoblast-like stem cells are present in several bilaterian lineages[68], their phylogenetic distribution is much more parsimoniously explained by convergent acquisition, rather than as an ancestral presence with multiple losses. Transcriptomic and genomic characterization of neoblasts in various animals may additionally reveal shared molecular signatures that also result from convergent acquisition. Also, the orthology of the position-control genes expressed by muscles during planarian and acoel regeneration has not been established[69], and therefore it's not clear if their role in regeneration is inherited from a common ancestor or not.

To date, regeneration studies on acoels have been mainly done in species belonging to the Bursalia suborder (e.g. *Hofstenia miamia*, *Isodiametra pulchra*). But, to our knowledge, regeneration power is not yet reported in the ca. other 400 acoel species nor in other Xenacoelomorphs (Xenoturbellids and Nemertodermatids)[73]. The example of sponges clearly demonstrates the intra-phylum plasticity of WBR and highlights the importance of studying more related models. This may be the case for acoels too, as they are known to evolve relatively fast[74] and to harbor many derived characters among Xenacoelomorphs, such as the organization of body muscles, or the presence of epidermal eyespots[75, 76]. Consequently, acoels alone cannot be taken as a proxy for

Xenacoelomorpha and ancestral reconstruction of bilaterian WBR will not be possible without exploring anatomical, cellular and molecular diversity across Xenacoelomorpha.

Despite these caveats, the comparison between acoels and planarians is highly relevant to reconstruct the ancestral mechanisms of WBR in Bilateria. It is important to note that this holds regardless of the position of acoels as the sister group of Nephrozoa or Ambulacraria, since in both cases the last common ancestor of acoels and planarians is the ancestor of all Bilateria (Fig. 2B).

3.3. Plastic families: convergent acquisition of WBR in tunicates

Increasing the phylogenetic resolution and comparing multiple closely related species is crucial to assign confidently the directionality of evolutionary transitions. Tunicates include so-called solitary species, where regeneration is limited to some tissue and organs[77] and colonial species, which are all able to undergo WBR via different types of budding[78]. Tracking WBR evolution in tunicates benefits from numerous anatomical studies on many species combined with well-resolved and robust phylogenies that allowed to infer multiple independent acquisitions of WBR in the whole subphylum[34, 78, 79]. For example, the evolution of budding in the family of Styelidae remained largely speculative until recently. Berrill[80] considered that all colonial species belonging to this family should be unified as a natural group because he assumed that they all perform the same kind of budding. In contrast, Kott suspected that budding modes may be more diverse than expected and advocated for “*accurate resolution of their taxonomy [and] information on the process of vegetative reproduction*”[81]. Recent phylogenetic reconstruction of Styelidae[34], as well as a closer look at the budding tissues in the species *Polyandrocarpa zorritensis*[82]

showed that the fundamental differences in the mechanisms of bud formation, as well as their phylogenetic distribution, are more parsimoniously explained by convergent acquisition[34]. Thus, according to these data, three modes of WBR have been independently acquired (Fig. 2C) from a solitary, non-budding, ancestor of Styelidae. Therefore, the question is to know whether homologous modules (e.g. GRN made of orthologous genes) have been convergently deployed in these three non-homologous budding modes. The discovery of such shared GRN or budding cell types between the different budding modes in Styelidae will be interpreted as independent co-options, as long as the phylogenetic topology makes the convergent acquisition of budding the most parsimonious hypothesis.

3.4. A roadmap to investigate WBR evolution

These three examples clearly show that, in the attempt to infer the evolution of regenerative phenomena, the phylogenetic relationships between the considered organisms must be used as an interpretative framework to formulate hypotheses on evolutionary trajectories. Then, each defined character should be first considered independently (e.g. presence/absence of neoblasts, expression of Wnt orthologues, a given morphogenetic movement, etc.) in order to reconstruct the mosaic of derived and ancestral states that make up the regenerative process and its phylogenetic distribution. Combining several lines of evidence such as histology, morphology, molecular signatures (e.g. by RNAseq) and phylogenetic analyses of genes of interest is, therefore, an informative way to refine homology hypotheses. When possible, multiple species must be considered in parallel to cover the diversity of the regenerative mechanisms (including absences) across the studied taxa. Finally, a particularly informative ontogenetic step to

collect characters related to regeneration may be the earliest steps after the injury, at the interface between the wound healing (when present) and the mobilization of the precursors (i.e. stem cells or dedifferentiating cells). For instance, recent RNA-seq and ATAC-seq analyses on fine-grained time series have shown that several species of bilaterian and cnidarians overexpress *immediate-response genes* such as EGR or Runt homologs, and establish Wnt signaling centers at the onset of regeneration[11, 83–86]. However, Wnt genes expressed in different regenerative contexts across species are not orthologous and are likely under the control of non-homologous mechanisms[83]. This and the patchy distribution of WBR may point toward an evolutionary scenario where WBR arose multiple times independently during metazoan evolution, often reusing similar modules co-opted from embryogenesis (e.g Wnt canonical pathway) while also assembling original modules specific to each regenerative strategy.

4. What is the significance of WBR? An integrative and practical approach

Regardless of the phylogenetic context - single or multiple acquisitions/losses of regenerative capacities - the advantages of regenerating a large portion of the body, or of multiplying individuals by budding, might seem self-evident. **These advantages** were largely assumed by early scholars, as Reaumur[87] and Bonnet[88], long before any theorization of evolution by means of natural selection. Yet, trade-offs between costs and benefits of regeneration might exist - and sometimes the benefits themselves might be difficult to identify, as in the case of the constant cycles of zooids destruction and regeneration in the colonial ascidian *Botryllus schlosseri*[89]. The challenges in

understanding the evolution of WBR among metazoans depend thus also on the difficulties in answering an apparently elementary question: what are the consequences of regeneration on the survival and/or reproductive fitness of an individual? In other words, is regeneration, or the loss of it, adaptive?

Following Darwin's work[90], Weismann explicitly regarded regeneration as an adaptive phenomenon "*the degree to which it is present is mainly in proportion to the liability of the part to injury*"[7]. Morgan, who was skeptical of untested theoretical explanations, set out to validate experimentally this prediction. In order to test whether the regenerative potential of a body part correlated with its risk of being injured in nature, he chose as a study model the hermit crab *Pagurus longicarpus* (Fig. 1F), as its anterior appendages were exposed to damage, while its posterior ones were "*naturally protected*" by the host gastropod shell. All appendages proved to regenerate well, which led Morgan to reject any adaptive value for regeneration. Morgan's experimental setup was however criticized for oversimplifying the parameters of the problem. Needham, in particular, argued that for a correct estimation of the evolutionary pressures, the "*indispensability*" of each appendage had to be considered. After recapitulating the experiments on *Pagurus*[91] and other crustaceans[92], Needham remarked that, *i*) the frequency of regeneration in posterior, more protected, appendages was indeed lower (in *Pagurus* it was 21 % vs. 83 %), and that *ii*) each pair of posterior appendages was essential to locomotion (and thus for survival of the crab). Thus, not only there was a correlation between risk of injury and regenerative potential, but the maintenance of a complete pair of posterior appendages was likely under strong selective pressure, supporting the old idea that regenerative abilities had an adaptive value[92]. The question was thus far from being settled because

if purely adaptive interpretations could explain the patchy distribution of regenerative potential among metazoans, it remained difficult to account for the similarities among regenerative processes[8]. Goss crystallized this idea and argued that if regeneration was truly an adaptive phenomenon, it must have arisen (and been positively selected) from non-regenerating ancestors multiple times, which would entail substantial differences between developmental mechanisms[8]. Shared features between diverse regenerative processes had instead been demonstrated, such as the requirement for innervation[93, 94]. Previous research had further highlighted a certain degree of similarity between embryonic and regenerating limbs, notably concerning patterning[95, 96] and morphogenesis[97]. Goss, like Morgan, favored a scenario where regeneration would be an inherent feature of metazoan life, and most likely a derivative of a core embryonic developmental program[98].

In his view, the modern phylogenetic pattern of regenerating taxa could be interpreted as the result of repeated losses of potential - themselves the consequence of other adaptive processes, for instance, the evolution of better brains in vertebrates[99], or the transition from aquatic to terrestrial habitats[8]. While some similarities among regenerative processes do exist, for example with regard to wound healing[100], it is today clear that the comparison is far from being trivial, as also concluded by Morgan, and that the answer cannot derive from the “mere” addition of further, diverse types of data. The previous examples on sponges, flatworms, and tunicates show that the identification of the relevant comparisons, at all the **different** scales, is key. Regarding the shared features of regeneration and embryogenesis, for example, recent transcriptomic approaches have indeed highlighted some degree of conservation in sequential gene usage between

embryonic processes and regeneration[101–104]. On the other hand, regeneration is broadly thought to display specific features, such as an involvement of the immune response[105], of the nervous system[106], and perhaps of muscle cells[69].

4.1 The puzzle of “restriction and absence” of WBR: eco-evo-evo perspectives

Representatives of sponges, acoels, planarians, tunicates but also cnidarians, ctenophores, annelids, echinoderms, and placozoans display different WBR capacities. The ability to regenerate large portions of the body is conversely lacking in arthropods, which nevertheless can regenerate their appendages until they reach a terminal molting stage - suggesting a possible trade-off between a protective cuticle and WBR, probably emerging at the origin of Ecdysozoa[107]. The problem with the “restriction and absence”[108] of regenerative potential among taxa remains central to the study of the evolution of regeneration[4]. As highlighted in the previous sections, the fragmentary taxonomic sampling is a major limit in understanding the evolutionary trajectories of WBR. The absence of regeneration is particularly difficult to address, and any explanatory research would need to take into account three parameters:

- i. *Evolutionary parameters*, in the form of a robust and well-resolved phylogeny for discriminating between putative losses and *de novo* acquisitions;
- ii. *Developmental parameters* - for example, taxon-specific anatomical features, such as the cuticle of ecdysozoans.

- iii. *Ecological parameters* - for example, the presence of environmental turbulence, such as the wind-generated waves fragmenting naked coral embryos after mass spawning events[109].

Habitat, body size, reproduction modes, anatomy, defense mechanisms might all be factors to consider. The intersection of ecological, developmental, and phylogenetic parameters poses a methodological challenge, and an eco-evo-devo approach has the potential for providing a common framework for tackling the issue[110].

Recent works have extensively discussed the ultimate causes of a reduced regenerative potential[4]. **These works argue** either that some selective pressure could play against the preservation of regenerative capacities, or that no particular pressure would maintain it, so that it becomes a neutral trait. The studies directly addressing the ultimate causes of regeneration are rare. A famous example is the loss of regenerative capacity in some groups of spiders, including the black widow (*Latrodectus mactans*). Spiders usually regenerate well their injured legs[111], with the notable exception of few orb-weaving genera, where it has been hypothesized that a regenerated appendage could impair web-making more than a missing one[112]. In this case, a strong pressure, the need for a geometrically accurate spider-web, selected against the maintenance of regenerative capacities. Conversely, if no particular pressure maintains regenerative capacities, for example, if predation is low[113, 114], these could be lost. Neutrality could also emerge if regenerative phenomena were essentially a by-product (an epiphenomenon) of other developmental processes under selective pressure and if the molecular link between modules was lost, for instance due to the activity of selfish genetic elements[115]. Continued tissue growth[116], agametic reproduction, or core embryonic

mechanisms[117] have all been proposed as processes from which regeneration might have derived.

A taxon-restricted loss of regenerative capacities does not necessarily imply an elimination of the genetic program for regeneration. Are there any latent or inhibited regenerative capacities in taxa that usually do not display them - and which could thus be re-activated? In naidine annelids, both comparative regeneration experiments and phylogeny indicate multiple events of loss of head regeneration. Interestingly, in one species, amputation during asexual fission within a small proliferative region harboring activated stem cells could elicit regeneration of a normal head[118]. This indicates that, despite the loss of regeneration, the capacity remained latent in these annelids, and could be re-activated. This study is a further reminder that a comparative experimental approach is essential for understanding the evolutionary trajectories of regeneration.

The problems with the loss of regenerative capacities, its significance for the fitness of organisms, and the question of whether regeneration is an attribute of all organisms are not purely theoretical. Indeed, our hopes of inducing regeneration where it does not occur, for example in adult humans, ultimately rests on the assumption that potential for regeneration might remain latent in organisms who are currently unable to do it[119].

4.2 Questions and approaches to investigate WBR evolution

Regardless of the evolutionary scenario, WBR constitutes a particular category of regenerative phenomena, whose links to physiology and reproduction are blurred. Here we have considered WBR in its most inclusive sense, including physiological regeneration and asexual reproduction, and effectively adopting the functional definition of

regeneration that - by replacing essential body parts - significantly delays an organism's death. But how to practically study WBR, placing this phenomenon in its evolutionary, developmental and ecological context? The questions raised through the Weismann vs. Morgan adaptive/innate debate are still highly relevant today. The criteria and strategies then proposed can represent today the starting points for practically shaping an integrative research program on the complex issue of whole-body regeneration.

i. Does the regenerating structure/body part experience frequent injuries in nature?

Injuries, for example, due to sub-lethal predation, are frequent in marine invertebrates, either planktonic or benthic[113, 120, 121]. In several demosponges, the rate of regeneration was shown to vary across species and to be inversely correlated to the frequency of injury[122], and, interestingly, not to be a consequence of phylogeny or physiological growth rate[122, 123].

ii. Is functionality fully recovered after WBR? Restoration of key functions, more than

a perfect "replica" of the missing parts, is necessary for survival, as also identified by Needham[91]. The hydrozoan jellyfish *Clytia hemisphaerica* has recently been shown to efficiently recover buoyancy and feeding after large injuries, while the original body symmetry is not necessarily restored [124].

iii. Is WBR significantly expanding the life-span of an organism? Arguably, recovering

from large injuries extends an organism's life expectancy, but other phenomena can be considered. The physiological regeneration of the colonial ascidian *Botryllus schlosseri* represents an interesting case, where the succession of generations could contribute to eliminating the senescence of the single

individuals, rejuvenating the tissues but preserving the pre-adaption of the colony to its surrounding environment[125]. An alluring corollary to this argument is that regeneration time is expected to be significantly shorter than the life-span of an organism.

- iv. How is WBR ecologically relevant? This question needs to consider that organisms are affected by their environment, and in turn, they modify it. Increasingly frequent episodes of mortality sweep through marine ecosystems due to extreme climatic events[126], which locally destroy benthic communities. The strategies of recovery, for example between marine invertebrates undergoing WBR or recruiting new larvae have a key impact on the dynamics of the benthic community. With regard to the second point, annelids have an important biogeomorphic impact on marine sediments, and regeneration negatively impacts their reworking of sediments[127]. Additionally, as WBR is tightly linked to the production of new individuals, it might represent a dispersal strategy[128], allowing organisms to colonize rapidly a novel or changing habitat, as it has been shown for forest recovery after fires[129]. The consequences of WBR on the invasiveness of a species and perhaps on the emergence of new species following reproductive isolation have been poorly studied, but constitute an interesting avenue for future research.

The extreme nature of WBR poses unique challenges, in particular when we try to investigate and measure the ecological and physiological implications. The resources required during WBR cannot be made available to other processes [130] This suggests important trade-offs for the organisms concerned, which need to be identified and

quantified. These trade-off concern the regenerative events, but also the loss of a body part itself. With regard to the cost-benefits of the regenerative process itself, regeneration subtracts resources from growth and reproduction, the so-called regenerative load[131]. In sponges and corals, injuries inflicted when food is scarce or when the animal had been previously injured regenerate less well, showing that resource allocation is critical[132]. On the other hand, besides the obvious benefit in avoiding looming death, WBR might provide some specific advantages, for example, a rapid adaptation to changing environments[125, 133]. In heteromorphic colonies of hydrozoans and bryozoans, changing environmental conditions could cause the regression of existing individuals and the generation of a different type of specialized zooid[125, 134]. Interestingly, given the colonial nature of these organisms, the costs of the process would be reduced by the re-utilization of regressing individuals[20] as a source of materials and energy for the growing ones.

The loss of body parts is more difficult to quantify. Energy loss is a multifaceted variable, but the dry weight of the removed body part has been used as an estimate[135]. Short-term, acute, costs include the loss of foraging or motility, of body mass, risk of infection, behavioral disruption, impaired self-, and non-self-recognition; while lower fecundity or growth (due to loss of germ cells or energy storage) might be seen in the long-term[136]. The loss of an arm, for example, has a greater cost for asteroids than for crinoids or ophiuroids, as they bear gonads[135]. As for the eventual benefits, it might seem difficult to imagine any advantage in losing a body part. Yet, autotomy, the active breaking of a body part along a predetermined “plane”, suggests a possible scenario: crustaceans, annelids, holothurians and other animals shed body parts as a defense mechanism, in

order to escape predators or to isolate infected or malfunctioning body parts (reviewed [137]).

5. Conclusions

When, later in his life, Morgan heard that a 24-year-old Norman John Berrill was working on marine worms and ascidian development and regeneration, he reproached him saying “*You are being very foolish [...] At your age you cannot waste your time. We will never understand the phenomena of development and regeneration.*”[138]. Perhaps, if he had access to the theoretical tools of eco-evo-devo and to the technological resources available today, he would have thought otherwise. Morgan’s emphasis on exploring the vast diversity of both developmental and regenerative phenomena, and experimenting with testable hypotheses in models, represents the assets of his legacy. The very same *modus operandi* could help to avoid hasty interpretation and to remove anthropomorphic biases in how we interpret natural phenomena. Luckily, the young Berrill did not take Morgan’s advice and “[...] *continued watching in wonder to my heart's content and I am even more bewildered, though more sophisticated, by what I see*”[138].

6. Acknowledgments

The authors want to thank Evelyn Houliston, Lucas Leclere, and Elena Casetta for their valuable comments on the manuscript. This work was supported by ANR (ANR- 14-CE02- 0019- 01).

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8. Figure legends

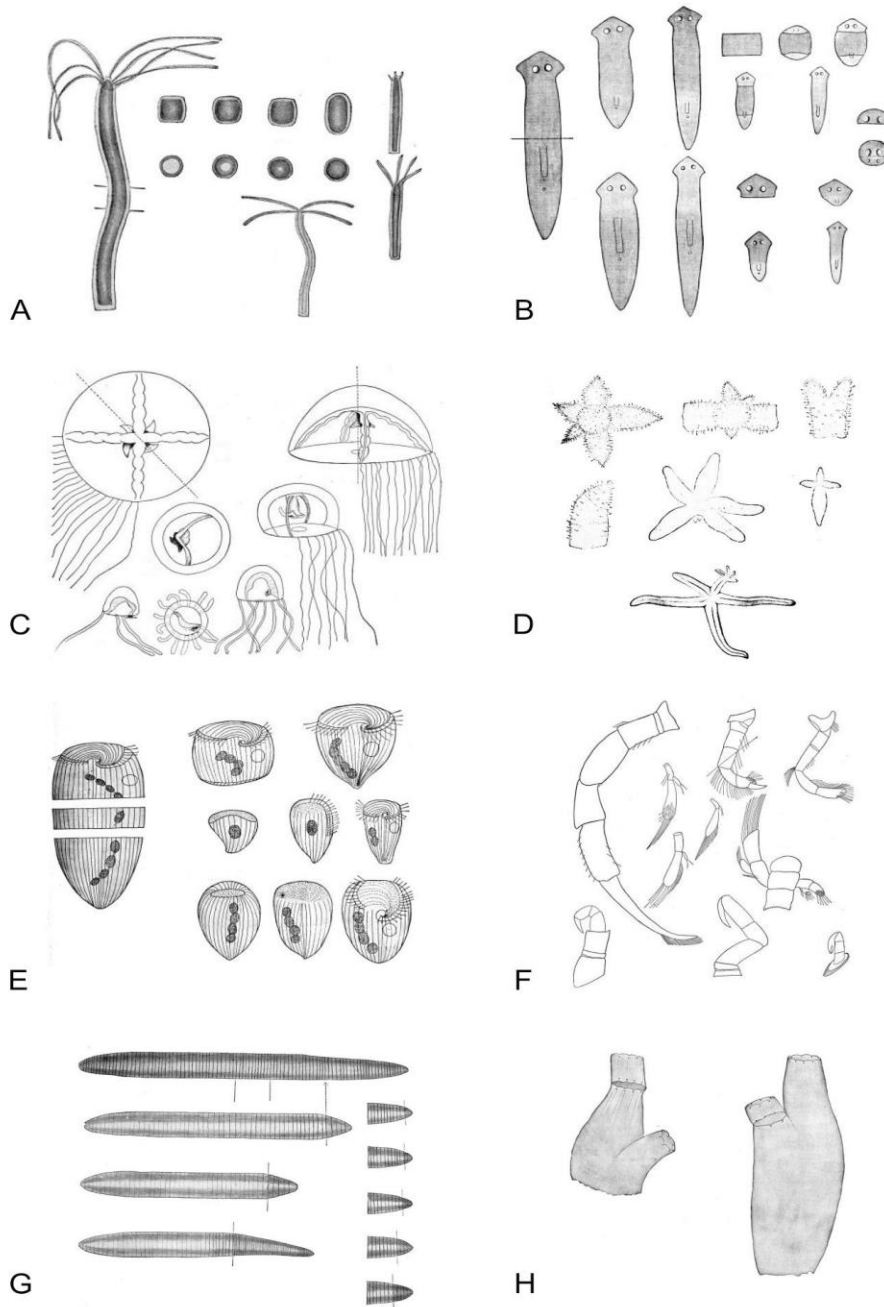


Figure 1. Example of regenerating animal models reported in Morgan's *Regeneration* (1901). **(A)** *Hydra viridis*, **(B)** *Planaria maculata*, **(C)** *Gonionemus vertens*, **(D)** *Linckia multiformis*, **(E)** *Stentor coeruleus*, **(F)** *Eupagurus longicarpus*, **(G)** *Allolobophora foetida*, **(H)** *Ciona intestinalis*. Modified from Morgan (1901)[1].

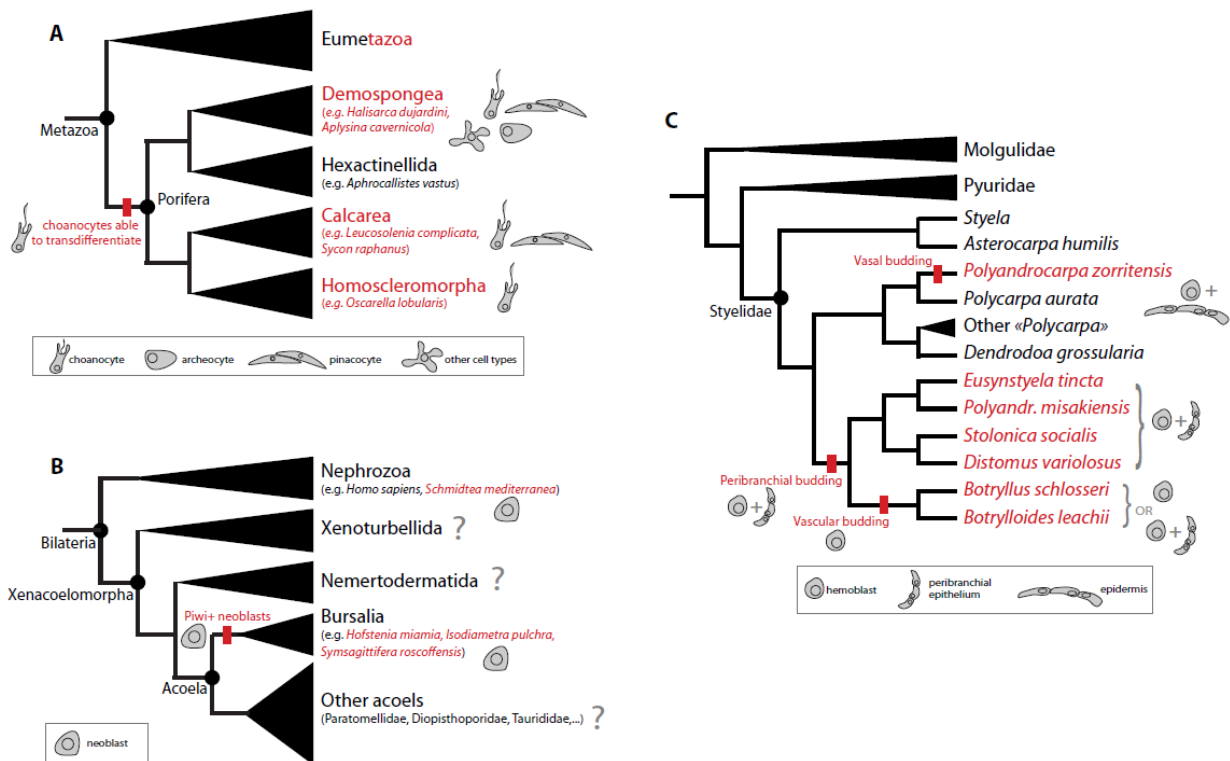


Figure 2. Phylogenetic relationships between species cited in the text, and cell types involved in WBR, in sponges **(A)**, xenacoelomorphs **(B)** and ascidians **(C)**. The species that are reputed for their extensive ability to regenerate are represented in red. The cells

drawn represent the cell types known to supplement more tissues during regeneration, by proliferation and/or differentiation. On branches are shown ancestral reconstruction regarding the role of each cell type in WBR based on parsimonious optimization.