

Phylogenic Comparison of Epimorphic Regeneration and Wound Healing

Introduction

Every animal species has the capability to heal from injuries (Miguel-Ruiz and Garcia-Arraras 2007). Methods used range from replacing the lost cells with undamaged stem cell progenitors, proliferation and migration of tissue cells near the wound, transdifferentiation, dedifferentiation and repatterning, and more (Thouveny and Roy 1998). All types of repair that replace damaged cells would be considered regeneration. It is obvious what advantages regeneration would provide any animal, leading to the evolution of various niche derived mechanisms. Of the evolution of these regenerative systems, the most consequential and controversial seems to be epimorphic regeneration, the use of dedifferentiation and repatterning (Kierdorf et. al. 2007).

There are two views of the evolutionary path epimorphic regeneration has taken. The first view, the *inherent* hypothesis, states that the ancestral animals all had epimorphic regenerative capability, but over the course of evolution some divergences lost the ability. The *adaptive* hypothesis, on the other hand, suggests that epimorphic regeneration today has evolved independently in species and is not an ancestral heirloom (Thouveny and Roy 1998).

The definition of epimorphosis has been the subject of much debate (Kierdorf et. al. 2007). The specific definition will be discussed later. But, with any of the various views on the process, organisms with epimorphic regenerative capability are found in many branches of the tree of life and are not all directly connected (Thouveny and Roy

1998). For almost every species that shows capability, there is an evolutionary theory explaining why they harbor this ability while closely related species do not (Goss 1992).

Several factors influence the debate between adaptive and inherent evolution. At the forefront are the intricacies of natural selection that could have lead to each case. The cases include not only the differences between larger groups of organisms, phylums or classes, but also include the differences in regenerative capability seen among species of a single genus (Wagner and Misof 1992). Also important to the debate are the specifics of the mechanisms, the processes or pathways used. The occurrence of epimorphic regeneration either adaptively or inherently implies certain commonalities or differences between the processes in each species it is seen in. Homologies between tissue patternings (Kierdorf et. al. 2007), genes activated (Thouveny and Roy 1998), pathways involved (Pearl et. al. 2008), and other cellular requirements (Goss 1992) are all strong factors that lean towards or away from either hypothesis.

Defining Epimorphic Regeneration

Before getting too far in to the intricacies of the process of epimorphic regeneration and the species that display it, it is important to define what makes regeneration epimorphic and what other possibilities there are. Although the term ‘epimorphic’ has been used since the beginning of the 1900s, the specifics of it are still debated today (Kierdorf et. al. 2007). For the discussion here, the definition used will be strongly similar to what Kierdorf et. al. (2007) settled on. For regeneration to be considered epimorphic, it must involve the dedifferentiation of cells at a wound site following wound healing and resulting in the formation of a blastema. The blastema is defined by its capacity to promote proliferation and organize pattern formation. This process can reconstruct appendages or body fragments with complex histology.

Kierdorf et. al. (2007) broaden their definition to not require that the blastema form from dedifferentiation. This allowed epimorphic regeneration to also encompass antler regeneration in deer. I specifically make the requirement of dedifferentiation because it suggests a process that is more intricate than a simple reinstitution of embryonic development. Also, the stereotypical examples of epimorphic regeneration, i.e. urodeles (Thouveny and Roy 1998), teleosts (Nakatinik et. al. 2007) and others, display dedifferentiation, while the more controversial models, like deer antlers, do not display it. With regards to the evolution of regeneration, it is likely simpler to evolve a non-dedifferentiating regeneration, as perhaps an expansion on scar formation (Goss 1993), than to evolve a signaling pathway that moves cells backwards on the differentiation pathway. Non-dedifferentiating regenerations may thus be artifacts in picture of the evolution of epimorphic regeneration.

This definition does not require or even imply that the process of epimorphic regeneration must resemble a resurgence of embryonic development. Although in some species it is generally accepted that very many of the same pathways and structures are used in both (Thouveny and Roy 1998), as will be discussed later, there is not enough data on all of the species that portray epimorphic regeneration to make the connection on a broad scale. Thus, the comparison of embryonic pathways to regenerative will not be discussed at length unless there is some sort of play, such as induction or inhibition, between the two. In the species where regeneration only occurs in the still developing organism, a clear distinction will have to be made between the processes if possible.

Epimorphic regeneration and 'wound healing' have very much in common. In anuran limb regeneration, wound healing takes place in the first few days after amputation, producing the wound epithelium (Thouveny and Roy 1998). Both wound healing that leads to regeneration and wound healing that does not result in regeneration have similar processes. These include inflammation, blood clotting, wound epithelium formation, fibroblast migration, ECM reconstruction, MMP upregulation, fibroblast migration, and nerve dependence (Roy and Levesque 2006). After these processes the two diverge. Wound healing concludes with scar formation while epimorphic regeneration continues on to dedifferentiation and blastema formation and proliferation. While the rate and specific weight of each process may differ between species, the general systems hold throughout.

Early Phylogeny of Regeneration

The earliest multicellular animals are all presumed to have mechanisms of regeneration. Provided here is a brief overview of regeneration in the invertebrates. While an understanding of the spectrum of regenerative capability across the invertebrates is important to the determination of the evolutionary mechanisms in play, the true testament will be of the higher ordered, more complex organisms. Figuring out how epimorphic regeneration was to occur in both the early invertebrates and the later vertebrates is more the focus of this paper. The homology of the regenerative process across much of the invertebrates is not receiving much debate.

There are correlations though, that are seen in modern species with regards to regenerative capability. Since these stem from the early examples, it is important to consider them. The main correlation is the general decrease of regenerative capability as the organism increases in complexity (Tsonis 2000). Since the term 'complexity' can be sometimes misconstrued and it is difficult to define a set 'complexity value' to an organism, this correlation can also be seen as a decrease in regenerative capability coinciding with an increase in size, patterning, or evolutionary distance. Also, when looking through the invertebrates, it will be important to make note of what kind of adaptation the regenerative capability is. The original ability of multicellular organisms to proliferate is an undebated adaptation. But, regenerative capability, especially when considered at all levels from simple proliferation to complex epimorphic regeneration, can be an adaptation, exaptation, disadaptation, or nonadaptation. And most of these can be subdivided into primary or secondary. These terms are defined appropriately by Baum and Larson (1991).

Cell proliferation goes hand in hand with multicellularity. Regeneration is different from normal growth in that it is the result of damage to the organism. In the original multicellular organisms, regeneration may have been the method of replacing damaged cells, but it doesn't have to be. That hypothesis considers original regeneration to be an adaptation for dealing with cell damage and death. While very probable, this isn't the only purpose that the mechanisms of regeneration can serve in an organism. As you will see, many early species reproduce asexually, employing the same pathways that are used in regeneration (Alvarado 2000). In this instance, regeneration could be an exaptation. The specific ways that the mechanisms for regeneration can be selected for will be discussed later. Now, we look at the history of the behavior.

Some 1600 million years ago animals emerged as distinctly different organisms from plants and then fungi in the evolutionary tree (Wang et. al. 1999; Wainright et. al. 1993). The earliest animals to take a distinct hold on the environment, and not be lost through time, were the unsymmetrical Porifera and the symmetrically radial Cnidaria (Knoll and Carrol 1999). Here, at these earliest stages of animal evolution, we see why the definition of epimorphic regeneration is very important. Porifera all exhibit regeneration, but they do not require dedifferentiation. Archeocytes, multipotent cells acting like stem cells, form a blastema after injury, but there is no dedifferentiation. The cells necessary for regeneration are already present (Thouveny and Roy 1998). Porifera are also an example of an organism that can regenerate an entire organism from a fragment, but considering them as an organism of colonial single cells, this is far closer to simple proliferation than regeneration. Coelenterates, encompassing the closely related Cnidaria and Ctenophora, are our first examples of species that reproduce by budding,

using a structure histologically similar to a blastema. After an injury, they have many options available to them, including dedifferentiation as in epimorphic regeneration, or transdifferentiation, a morpholactic response, or proliferation stem cells as Porifera do. While all Coelenterates are capable of these regenerations, the main focus in the phylum is on Hyrdazoa, because of their expected place in phylogenics, as a common ancestor to most metazoan species (Galliot 2005). Hydra are difficult to classify when it comes to regenerative behavior. They can regenerate any structure, but do so by only transdifferentiation. When replacing lost structures, they do not gain any cells. Though, at the same time, Hydra are constantly undergoing proliferation to replace cells naturally shed. So, after amputation and regeneration the eventually grow back to their former size, the mechanism is just not connected directly to injury response. They can also generate a full organism after fragmentation (Alvarado 2000).

The next phyla to evolve, Platyhelmenthes and Nemertea (Knoll and Carroll 1999; Alvarado 2000), and closely related Annelids (Thouveny and Roy 1998) all show extensive regeneration. They can almost all regenerate whole new organisms after fragmentation, as shown extensively in the model organism Planarians, a Platyhelmenthes in which there are a number of species that can regenerate from fragmentation but cannot reproduce by fragmentation, like most (Alvarado 2000). But, while Platyhemmenthes utilizes stem cells and does not always display dedifferentiation, they other two types have no totipotent cells on a normal basis, and undergo full epimorphic regeneration when injured. They do not always go through the whole process though; if an annelid can get away with just transdifferentiation and proliferation

than it will. It is also likely that Platyhelminthes cells can undergo dedifferentiation, they simply do not need to (Thouveny and Roy 1998).

Following the unsegmented worms (Knoll and Carroll 1999), Molluscs show epimorphic regeneration of a number of organs, but not all. Some damage does not require dedifferentiation, such as damage to the shell, but they are known to display the standard pathway of wound epithelium formation, dedifferentiation and blastema formation, and the repatterning and proliferation successfully (Thouveny and Roy 1998).

Arthropods have even less of a capability, and with far more restrictions. The regeneration they do undergo is epimorphic and is usually of the legs. In some species though, leg regeneration rate is positionally dependent. In most cases, regenerative ability has found to be dependant on molting in adults (Thouveny and Roy 1998; Goss 1993). Arthropods are a very important case when it comes to determining the origins of regenerative capability. Much like arthropods, higher chordates also only have the tendency to regenerate appendages, but the two do not share any ancestor's with appendages (Alvarado 2000). This will be discussed further later.

Echinoderms, as is well known, can regenerate fully from a fragment. They also display evisceration, autotomy of internal organs, after which they regenerate the missing parts. While not always required to apply dedifferentiation, these organisms do have the capability to utilize dedifferentiation and blastema formation when regenerating. It has also been seen in sea cucumbers that regeneration of different tissues involve the same factors (Miguel-Ruiz and Garcia-Arras 2007; Thouveny and Roy 1998; Alvarado 2000).

Lastly of the invertebrates are the lower chordates. They show regenerative capability by use of stem cells, and are also capable of reproduction by budding.

Transdifferentiation is commonly seen, but due to extensive involvement of stem cell-like cells dedifferentiation is not seen so much (Thouveny and Roy 1998).

Significance of Induced Regeneration

Here we take a pause from our journey through the tree of metazoan life to consider induced regeneration. There has been very little induction of regeneration in the invertebrates due to their extensive capabilities without it. But, in vertebrates, where our discussion is leading us next, there is an abundance of work on inducing regeneration in organisms or tissues that would not normally undergo the process. The specific examples of induced regeneration will be mentioned accordingly as we make our way up the phylogenetic tree. Here though, I would like to explain why induced regeneration is important to determining the evolutionary patterns of regenerative capability.

Methods of induction, such as innervation or embryonic grafts, are attempting to fill in the holes that have accumulated in the cascade of mechanisms representing successful regeneration due to evolution. They help to provide the pieces of the puzzle that is the complex interworkings of epimorphic regeneration. Once we know the components of the regenerative systems of successful animals, we can trace these components back to their ancestral roots. From there we can better understand where and why some species may have lost or gained the ability to regenerate complete structures. Also, by comparing patterns of components across species, we might be able to determine evolutionary distance from a regenerating ancestor. Knowledge today of the workings of regeneration is far too limited to provide any significant results in either of these areas, and it is beyond the scope of this paper to attempt anything without the

backing of crucial knowledge. So, we are limited to understanding that each successful induction experiment shows only that there is a connection, likely homologous, between the species that was induced, and the one that the induction mimicked.

Continued Phylogeny of Regeneration: Vertebrates

Among the vertebrates are the more complex examples and the more controversial in terms of evolutionary paths. Some of the earliest terrestrial vertebrates were Anurans and Salamanders, arriving between 200 and 400 million years ago (Kumar and Hedges 1998), allowing more than 150 million years of chordate evolution to come before them (Knoll and Carroll 1999). The Urodeles are the hallmark of epimorphic regeneration. They use epimorphic regeneration after any limb or tail amputation, and also with injuries to the upper or lower jaw. They also show regeneration of lens and retina, employing both transdifferentiation and dedifferentiation respectively (Campbell and Crew 2007; Morrison et. al. 2006; Thouveny and Roy 1998; Tsonis 2000). The urodeles are the most commonly used example of epimorphic regeneration, and so the process is the well studied and understood. The related order Anura, consisting of frogs and toads, also shows extensive regenerative capability, but not equivocal to urodeles. Many, but not all, species can epimorphically regenerate limbs, but the regeneration is heteromorphic, it does not result in an equivalent structure to the one lost. In the Larvae stage, these animals show more regenerative potential, such as that of the tail and early hind limbs. The capability to regenerate is more unevenly distributed among the anurans than the urodeles, with very few species actually achieving a regenerate limb as good as that amputated. It is still epimorphic regeneration because they go through the phases of

wound epidermis formation, dedifferentiation, blastema formation, and proliferation with re-patterning. The problem seems to occur with a failure to re-pattern correctly, resulting in a stump (Thouveny and Roy 1998; Pearl et. al. 2008). Regeneration in Anurans is a good example of a disaptation. Also, it is seen in Anurans that the extent of regenerative capability is not determined by the size or complexity of a species, as some larger frogs regenerate more completely than smaller (Thouveny and Roy 1998).

Reptiles, the evolutionary derivative of amphibians (Carroll 2008) are known for the regeneration of autotomized tails, a process which, while epimorphic is also heteromorphic. Limb regeneration does not occur naturally in reptiles. Inductions may be performed which show some success, but very little. In birds, where the chicken was used as a model organism, regeneration also does not occur. Also similarly, very poor regeneration can be induced in the developing chicken (Thouveny and Roy 1998). This induction is significant because it shows the decrease of regenerative ability, likely due to a decrease of necessary factors, coinciding with the evolutionary distance developing between birds which presumably evolved from reptiles 310 million years ago (Hedges 2002) and reptiles which as stated derived from their more regeneratively capable ancestors, the amphibians.

It is interesting that the most widely used model organism for studying regeneration in fish, the zebrafish, is expected to have evolved after the emergence of the land animals such as amphibians (Hedges 2002; Vanedpoele et. al. 2004). Interesting, but not truly significant as it is not under debate whether the ancestors of zebrafish had regenerative capability because extensive epimorphic regeneration is seen in many fish, but mainly teleosts. While not all species can regenerate equivalently, most can

reconstruct various fins through the use of dedifferentiation and a blastema (Wagner and Misof 1992). Telosts, including zebrafish, are another model system where extensive work has been done on their capability. Their epimorphic regeneration has been positively compared to salamander regeneration on many occasions.

In mammals, which stemmed from reptiles around 92 million years ago, epimorphic regeneration seen very sparsely, but does exist. Mammalian regeneration is of special interest due to the applicability to humans, who as we know have no epimorphic regenerative capabilities beyond the regeneration of the fingertips in children. Fingertip regeneration is also seen in other mammals, such as the mouse and monkey, but this capability is, as expected, highly regulated. There is also an interesting phenomena seen in mammals where some species have the capability to regenerate sections of their ears epimorphically. Rabbits and cats can regenerate small holes pierced through the ear. Bats can do this as well but can also do the same for holes cut into their wing membranes. Other mammals expected to be related to these examples do not show any capacity for regeneration (Thouveny and Roy 1998). This creates a conundrum when it comes to lining up the path of epimorphic regeneration as told by the inherent hypothesis. But, the close resemblance to other epimorphic regenerative behavior also puts strain on the adaptive hypothesis (Goss 1993).

There has been a lot of effort on determining whether the regeneration seen in deer antlers is epimorphic. It seems now that it does not fit the requirements laid out for use here, as it does not involve dedifferentiation. It does however involve the formation of a blastema, which promotes proliferation and pattern formation. The mechanisms behind antler regrowth have been compared with a form of 'super-scar formation'. While

this regeneration may be significant to understanding the patterns of selection that lead to regeneration, it is not expected to be directly connected to any other examples of regeneration (Kierdorf et. al. 2007). The explanation for the origins of antler regeneration would suffice to be a fully separate discussion. Here, the main focus is the path of epimorphic regenerative capability through the phylogeny.

The Selection of Regenerative Capability

As stated earlier, there are two main hypotheses when it comes to the appearance of epimorphic regeneration in higher metazoans. The adaptive hypothesis, which explains current models of regeneration as separate adaptations, and the inherent hypothesis, which explains them as all homologs of the ancestral ability to regenerate epimorphically (Goss 1992). Wagner and Misof (1992) looked at the evolutionary modifications of regenerative capability in teleosts, and found that there are three ways to explain a difference in capability between species. While their hypothetical reasons are not too far from each other, there is a significance in each one when relating back to the general hypotheses of adaptive or inherent. Wagner and Misof (1992) considered all of their specimen to have homologous behavior, the inherent hypothesis presiding. Here though, we see their three hypotheses as ways to explain either. Their first hypothesis was that differences in regenerative ability were due to *proximate* cause. That is, due to one of the necessary ingredients, be it nerves or hormones, has decreased enough to prevent regeneration. This is an example of a disaptation and of a puzzle piece falling out of place, which induction experiments try to fix. The Inherent hypothesis considers this cause more strongly because it offers the incidence of regenerative capability 'hiding' in

an organism until evolution selects for regeneration again, and thus the return of the lost elements of the pathway. Their second hypothesis was of an *adaptational* cause. This suggested that loss (or gain) of regenerative ability held some sort of selective significance and natural selection caused it to be removed or reinvoked in a species. The adaptation of regenerative capability can work with both the Adaptive and Inherent hypotheses. The Adaptive hypothesis suggests that the mechanisms for regeneration occur *de novo* while the Inherent hypothesis suggests that they are homologous.

Wagner and Misof (1992)'s third hypothesis was of *epiphenomenal* interaction. The loss (or gain, but rarely) of regenerative capability was a side effect of some other adaptation that was selected for. This cause stands in the way of the Inherent hypothesis most of the time, because it is difficult to redevelop regenerative capability in the same way once it has been interrupted, and it gets more difficult as the millions of years go by and bring about new adaptations that may also interact with the regeneration mechanisms. Both *epiphenomenal* and the *proximate* cause models are likely to occur when regeneration is no longer being selected for.

The Adaptive hypothesis does not require that all examples of regeneration be the result of independent evolution. It allows for regenerative capability to be carried through the ancestry of an organism and to result in its presence today and numerous ancestors of the organism (Goss 1998). This is what makes it nearly impossible to disprove. The linchpin of the argument is that regenerative ability has evolved independently at least once in the history of metazoan evolution. Any number of examples of homology does not disprove the hypothesis. The opposite goes for the Inherent hypothesis. The linchpin of this argument is that every instance of epimorphic

regeneration seen today has been derived from a common ancestral mechanism. It takes only one example of non-homologous epimorphic regeneration in animals to disprove the theory as a whole. Obviously, each hypothesis can still apply to individual branches in the evolution of animals if they fail as a whole, which will still be significant in the understanding of regeneration. But, although the weight of the Inherent hypothesis hangs on a proverbial thread, it has yet to be disproven as a whole. All examples of epimorphic regeneration in animals today shows enough homology to satisfy the argument.

Thus, the occurrence of a new mechanism of epimorphic regeneration can occur fairly early in metazoan evolution, which is why do discuss the correlations between higher vertebrates, it is important to show all of the examples in even the simplest invertebrates. Homologies between, not only the actual regeneration structures of the organisms, but of almost all cellular characteristics need to be taken into account. The most important example is that of the insect legs offered by Alvarado (2000). As stated earlier, insects and amphibians have no common ancestors that have appendages, but most examples of each retain the ability to epimorphically regenerate only their appendages. At first glance it seems like clear support of the Adaptive hypothesis. The legs of insects and tetrapods are clear indications of convergent evolution and are not homologous structures. But, the regenerative capability seen in the legless ancestor of both insects and tetrapods may have been such a mechanism that when appendages evolve, they have the tendency to regenerate more easily than the body. In this image, the legs of insects and tetrapods are still not homologous, but the molecular and cellular mechanisms driving their presence in the evolutionary tree are. It is going to take a lot of puzzle pieces to determine which is the case.

Homologies, Present and Otherwise

A homologous mechanism, such as the kind the Inherent hypothesis relies on, is determined by its derivation from an ancestral state. To be homologous, two mechanisms must show strong similarity to the same part of the ancestral mechanism. Cellular and molecular mechanisms today that are homologs are determined based on morphology and molecules involved. Comparisons can be made on either scale to the same effect.

Homology in regenerative mechanisms in early metazoans is not strongly debated. With such simple structures, as the hydra or the planaria, it is not a long shot to say that the mechanisms are related. The original morphological homology stems from use. Budding, a common method of asexual reproduction studied extensively in hydra (Galliot 2005), is homologous to budding seen in other cnidarians related species. Regeneration after fragmentation is another mechanism that seems to imply homology because of its extensive use among early metazoans (Alvarado 2000). Both of these examples, budding by reproduction and fragmentation stem from the original mechanisms of development established for multicellular animals. If regeneration also stems from this behavior it is likely homologous across the board.

From early animals to complex vertebrates we see epimorphic regeneration using a blastema. And while 'blastema' is a fairly general idea, the ones that occur in animals all occur fairly similarly histologically (Nakatani et. al. 2007). There cannot be many comparisons though, between the morphology of regeneration in early animals and late, due to the striking differences in structural similarities. Generalizations are applied perhaps a bit too often. The result of different evolutions of a similar ancestor, though, can be just as much help, because it can lead you to looking at the appropriate things

within the ancestor. As how most animals can regenerate their nonhomologous appendages. Regeneration of certain structures is a big part of what suggests homology. But, it is difficult to compare pattern formation in creatures that do not form nearly the same patterns. These patterning problems are sometimes made up for in the induction of regeneration in a more complex organism by copying what is seen in a simpler system.

It tends to fall down to the molecular pathways to represent homology between two cellular processes. Organisms tend to retain significant similarity in their molecular pathways, thus the origins of homology. New pathways cannot randomly generate successfully as species evolve—a point that the Inherent hypothesis is based on. Take for instance that many evolutionary trees, such as that of Wada and Satoh (1993) and Wang et. al. (1999), which use DNA sequence similarities to determine the evolutionary distance between organisms. If great DNA changes could occur randomly without a negative penalty on the organisms survival, there would be no chance of developing evolutionary trees in this fashion.

There are many molecular pathways that are still used today by both early invertebrates and later vertebrates. The similarities between regeneration based pathways are usually the result of most epimorphic regeneration being a recapitulation of embryonic development (Brockes and Kumar 2005), which has kept a great deal of its similarity over hundreds of millions of years. For instance, the Wnt/ β -catenin pathway seen in hydra regeneration (Galliot 2005) is homologous to the same pathway used in much development and regeneration throughout animals (Gilbert 2006). Also, interestingly, retinoic acid can be seen to have an effect on regeneration in animals it was tested with, suggesting that RA acceptors have also been carried on throughout animal

evolution and are used for patterning or proliferation (Tsonis 2000). These things alone do not show the homology across today's models of regeneration.

The true test lies in comparing species whose evolution diverged long ago. Unfortunately the work on the regeneration in some phyla is lacking. Due to its prevalence in medicine though, work on vertebrates is extensive. Regeneration across vertebrates is seen to have numerous factors in common, including FGFs, TGFs, EGFs and nerve dependence (Tsonis 2000). These similarities do show that there is definite homology across the vertebrates and such evidence strongly supports the Inherent hypothesis. But, no number of successes will prove Inherent over Adaptive until all of the puzzle pieces of epimorphic regeneration are put together.

Selective Pressures Affecting Epimorphic Regenerative Capability

Organisms are constantly evolving. Not so much as that it's impossible to see the homologies, but enough that it deludes the perfect picture of animal ancestry. Although birds and salamanders have strikingly different regenerative capability, it does not mean that they were so separate when birds first came about. Thus, between models of regeneration, the in betweens might no longer exist although they did at one time. Reptiles probably had strong regenerative capability when they first evolved, but lost it due to one of the causes discussed above. When a species moves into a niche that does not require regenerative capability they will most likely lose it. This obviously leads to the solution that early invertebrates, who have had more time to perfect themselves than more complex animals, must have a constant need for regeneration, as they do. So, the methods of regeneration in hydra or planarians when they evolved to create higher

ordered phyla were probably not the same as they are today. Perhaps at one time cnidarians only reproduced by fragmentation, and then learned budding after the separate lineages had been started. In effect, strong similarities between organisms that have had regeneration selected for for over 500 million years (cnidaria) and organisms that have had it selected for for only a few hundred million years (mammals) are probably not going to be blatant. Thus why each homology should be considered as very important.

A number of factors go into selecting for and against regeneration. Wound healing is considered primary among them because of the close relation between the two. Wound healing and regeneration in organisms that do both are very similar. Wound healing is also similar across species that do and do not regenerate. There is no strong debate on the homology of wound healing. Not because its more obvious, but because it is less useful. All species have methods of wound healing, which infers nothing special. It has been suggested that better wound healing could be selected for, causing a decrease in epimorphic regeneration (Goss 1992). This references the *apaptational* cause hypothesis. Reasons for this could include the wasted energy put into making a regenerated limb, or the inefficiency of a regenerating limb. Alvarado (2000) suggests that epimorphic regeneration may lead to cancer due to uncontrolled proliferation of cells, but organisms with epimorphic regenerative capacity do not have more cancers than those without, in fact they have less. It is possible that epimorphic regeneration can only exist when there is a high degree of pattern control, which may work against the formation of cancer. When the loss of epimorphic regenerative capability is detrimental it is more likely due to *proximate* or *epiphenomenal* cause. It is also possible that over time, as individual species have settled into their niches, if epimorphic regeneration is not

required it may be advantageous if the individuals that require epimorphic regeneration are wiped out (Goss 1992). Populations where epimorphic regeneration ran rampant may have been depleted because of their reliance on regenerating rather than avoiding injury. In salamanders, epimorphic regeneration may have been selected for due to their tendency to chew on each other when they are young (Thouveny and Tassava 1998), which is an interaction within the population and not between the species and potential predators or prey.

Selective pressures are well known to lead to convergent evolution, just as the insect and tetrapod appendage examples, and also as insect and bird wings. With regards to regeneration though, convergency is harder to determine because of its delicate pathways and widespread use in simple animals. To better understand convergent evolution of wound healing mechanisms we look at different kingdoms. Even though each has evolved the use of multiple tissue types, possibly homologously, the mechanisms of various types of regeneration in animals (Alvarado 2000) differ strongly from those of plants (Sanchesz-Serrano 2001) or fungi (de la Providencia et. al. 2004). They use different molecular pathways and different structural formations.

Works Cited

- Alvarado, Alejandro Sanchez (2000) Regeneration in the metazoans: why does it happen? *Bioessays* 22:578-590.
- Baum, David A., and Larson, Allan (1991) A Phylogenetic Methodology for Studying Character Macroevolution. *Systematic Zoology*. 40:1-18.
- Brockes, J.P. and Kumar, A. (2005) Appendage regeneration in Adult vertebrates and implications for regenerative medicine. *Science*. 310:1919-1923.
- Campbell, L.J. and Crew, C.M. (2007) Wound epidermis formation and function in urodele amphibian limb regeneration. *Cell. Mol. Life Sci.* 65:73-79
- Carroll, Robert L. (2008) Problems of the Origin of Reptiles. *Biological Reviews*. 44:393-431.
- De la Providencia, I.E., de Sousa, F.A., Fernandez, F., Delmas, N.S., and Declerck, S. (2004) Arbursular mycorrhizal fungi reveal distinct patterns of anastomosis formation and hyphal healing mechanisms between different phylogenic groups. *New Phytologist* 165:261-271
- Galliot, B. (2005) Regeneration in Hydra. *Encyclopedia of Life Sciences*. Doi: 10.1038/npg.els.0004186.
- Gilbert, S.F. (2006) *Developmental Biology: Eighth Edition*. Sinauer Associates Inc.
- Goss, R.J. (1992) The evolution of regeneration: adaptive or inherent? *J. Theor. Biol.* 159:241-260.
- Kierdorf, U., Kierdorf, H. and Szuwart, T. (2007) Deer antler regeneration: cells, concepts and controversies. *Journal of Morphology* 268:726-738.

- Knoll, Andrew H., and Carroll, Sean B. (1999) Emerging Views from Comparative Biology and Geneology. *Science* 284:2129-2137.
- Kumar, Sudhir and Hedges, Blair S. (1998) A molecular timescale for vertebrate evolution. *Nature*. 392:917-920.
- Miguel-Ruiz, J.E.S. and Garcia-Arreas, J.E. (2007) Common Cellular events occur during wound healing and organ regeneration in the sea cucumber *Holothuria glaberrima*. *BMC Devel. Biol.* 7:15
- Morrison, J.I., Lööf, S., He, Pingping and Simon, A. (2006) Salamander limb regeneration involves the activation of a multipotent skeletal muscle satellite cell population. *Journal of Cell Biol.* 172:433-440.
- Nakatani, Yuki, Kawakami, Atsushi and Kudo, Akira (2007) Cellular and Molecular processes of regeneration, with special emphasis on fish fins. *Develop. Growth Differ.* 49:145-154.
- Pearl E.J., Barker, D., Day, R.C. and Beck, C.W. (2008) Identification of genes associated with regenerative success of *Xenopus laevis* hindlimbs. *BMC Devel. Biol.* 8:66
- Roy, Stephane and Levesque, Mathieu. (2006) Limb Regeneration in Axolotl: Is It Superhealing? *TSW Development & Embryology* 1(S1), 12-25.
- Sanchez-Serrano, Jose J. (2001) Plant Responses to Wounding. *Encyclopedia of Life Sciences*. John Wiley & Sons, Ltd. www.els.net

- Thouveny, Yves, and Tassava, Roy A. (1998) Regeneration Through Phylogenesis (pp 9-43) *In* Ferretti, Patricia, (ed.) and Geraudie, Jacqueline, (Ed.) Cellular and Molecular Basis of Regeneration. John Wiley & Sons, England.
- Tsonis, Panagiotis A. (2000) Regeneration in Vertebrates. *Devel. Bio.* 221:273-284.
- Vandepoele, Klaas, De Vos, Wouter, Taylor, John S., Meyer, Axel, and Van de Peer, Yves (2004) Major events in the genome evolution of vertebrates: Paraneome age and size differ considerably between ray-finned fishes and land. *PNAS* 101:1638-1643.
- Wada, Hiroshi and Satoh, Noriyuki (1993) Details of the evolutionary history from invertebrates to vertebrates, as deduced from the sequences of 18S rDNA. *Proc. Natl. Acad. Sci.* 91:1801-1804.
- Wagner, G.P. and Misof, B.Y. (1992) Evolutionary Modification of Regenerative Capability in Vertebrates: A comparative study on teleost pectoral fin regeneration. *Jour. Exp. Zool.* 261:62-78.
- Wang, Daniel Y.C., Kuar, Sudhir, and Hedges, S. Blair (1999) Divergence time estimates for the early history of animal phyla and the origin of plants, animals, and fungi. *Proc. R. Soc. Lond. B* 266:163-171.