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SYMPOSIUM

Evolutionary Loss of Animal Regeneration: Pattern and Process

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Synopsis The ability to regenerate lost or damaged body parts is widespread among animals and provides obvious potential benefits. It is therefore perplexing that this ability has become greatly restricted or completely lost in many lineages. Despite growing interest in the cellular and molecular basis of regeneration, our understanding of how and why regenerative abilities are lost remains rudimentary. In an effort to develop a framework for studying losses of regeneration, here I outline an approach for rigorously identifying such losses, review broad patterns of regenerative ability across animals, describe some of the clearest examples of regeneration loss, discuss some possible scenarios by which regeneration may be lost, and review recent work in annelids that is providing new insights into loss of regenerative ability.

Introduction

The evolutionary loss of regenerative ability represents a fundamental and perplexing problem in biology. Although the complex question of how many times regeneration has evolved anew among animals remains to be resolved, it is evident that regenerative ability is widespread. Regeneration provides obvious benefits to an injured individual, yet regenerative abilities appear to have been greatly restricted or completely lost in many animal lineages (Bely and Nyberg 2010). Why should regenerative abilities ever be lost and how do such losses occur? Although regeneration has long fascinated humans (Morgan 1901; Dinsmore 1991), undoubtedly in part because of our own frustratingly limited regenerative potential, and although the process of regeneration has now been analyzed in considerable detail in a few model organisms (Sánchez et al. 2006; Carlson 2007; Brockes and Kumar 2008), our understanding of why and how regenerative abilities are lost (and possibly gained) through evolution remains extremely limited.

Developing a good understanding of the ultimate and proximate mechanisms of the loss of regeneration will require a targeted and multidisciplinary approach. It will be necessary to identify unambiguous losses of regeneration through comparative studies interpreted in a phylogenetic context, to reveal the ecological correlates and developmental basis of such losses, and to formulate and test specific models for how and why loss of regeneration occurs. Investigating a broad range of animals in this way will also be essential in order to assess the extent to which regeneration loss proceeds in a similar way across different groups.

As a step towards focusing efforts on this fascinating phenomenon, here I begin developing a framework for identifying and investigating regeneration losses in the Metazoa, review available data bearing on this topic, and suggest important avenues for future research. Specifically, I outline how to identify regeneration losses rigorously; review broad patterns of regenerative ability across animals; describe some of the clearest examples of regeneration loss; discuss some possible scenarios by which regeneration may be lost; and review recent data in annelids that are providing new insights. Although regeneration can occur at a range of biological levels of organization (cells, tissues, internal organs, structures) (Bely and Nyberg 2010), here I focus

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specifically on the regeneration of structures (e.g., limbs, fins, heads, tails), as loss of such structures occurs widely in nature and thus structure-level regeneration has a clear ecological and evolutionary context.

Identifying losses of regeneration

How to identify losses of regeneration

Identifying lineages that have lost regenerative abilities is an obvious first step in studying regeneration loss. Although loss of regeneration is thought to have occurred in many groups of animal and the optimal approach for recognizing such cases is straightforward in principle, regeneration losses have rarely been identified rigorously. Identifying losses of regeneration requires both thorough comparative experiments on regenerative ability and a robust phylogenetic framework for interpreting the pattern of the evolution of regeneration. Comparative experiments on regenerative ability should be performed on a range of species in the target group and homologous regions of the body should be removed in each species to ensure that amputations represent a comparable challenge across the species tested. If successful regeneration ensues, the presence of regeneration is demonstrated unambiguously. However, demonstrating the absence of regenerative potential is inherently problematic, since ''absence of evidence is not evidence for absence.'' If regeneration does not occur (or does not occur normally), further studies should be performed to confirm the inability of the species in question to regenerate successfully under a broad range of biologically relevant conditions. For example, amputations should be performed on individuals at different life-history stages (e.g., young/old, sexually immature/mature), of different nutritional status (e.g., starved/well fed), and under different environmental conditions (e.g., different temperatures) to determine whether the success of regeneration is merely a function of one of these variables. If such follow-up studies demonstrate that, rather than completely losing regeneration, a lineage has experienced a great narrowing in the conditions permissive of regeneration, this may itself be of considerable interest for understanding early stages of regeneration loss (see next section). Controls are especially important in trials of species suspected of not regenerating. Uncut individuals should be maintained to gauge the rate of mortality under the experimental conditions so comparisons can be made with cut animals. If it is possible to assess during the trial the investment by individuals into other developmental processes (e.g., growth, reproduction), this can also strengthen the case for the absence of regeneration. For example, if amputated animals continue to grow or reproduce during the trial, even as they fail to regenerate, this demonstrates that individuals have sufficient resources available for these other processes and that these resources can be mobilized under the experimental conditions.

Once thorough experiments on regenerative ability have been completed, the resulting data should be interpreted in the context of a robust phylogeny to reconstruct the pattern of evolution of regeneration. Although the loss of regeneration is generally assumed to be much more likely than the gain of regeneration, non-regenerating species should not necessarily be assumed to represent losses (Brockes et al. 2001); gains of regeneration, although poorly documented, are certainly possible. A loss of regeneration is most easily identified when non-regenerating taxa are nested well within clades that otherwise have the ability to regenerate. When the phylogenetic distribution of the presence/absence of regeneration makes the polarity of change less obvious, additional taxonomic sampling or further work on the developmental basis of regeneration (or failure of regeneration) may be necessary in order to confidently reconstruct the direction of evolutionary change.

In practice, the approach outlined above has rarely been followed. Thus, although a number of losses of regeneration have been tentatively identified, most still await rigorous confirmation. A number of challenges have hindered the identification of regeneration losses thus far. First, published information on regenerative ability remains limited. For many animal groups, even some entire phyla, there is simply no information on regenerative ability (Fig. 1) (Bely and Nyberg 2010), and if documentation is available, it is often available for only a tiny fraction of the species in a group. Furthermore, only a few studies have systematically performed studies of regenerative ability using comparable amputations across a range of related species (e.g., Scadding 1977, 1981; Vollrath 1990; Wagner and Misof 1992; Bely and Sikes 2010). Instead, most available data on the distribution of regeneration are gleaned from single-species laboratory studies (typically developmental or physiological studies) or field studies reporting naturally regenerating individuals, such that amputations and conditions are often inconsistent across species. Follow-up studies to confirm the absence of regeneration under a range of conditions are rarely conducted.

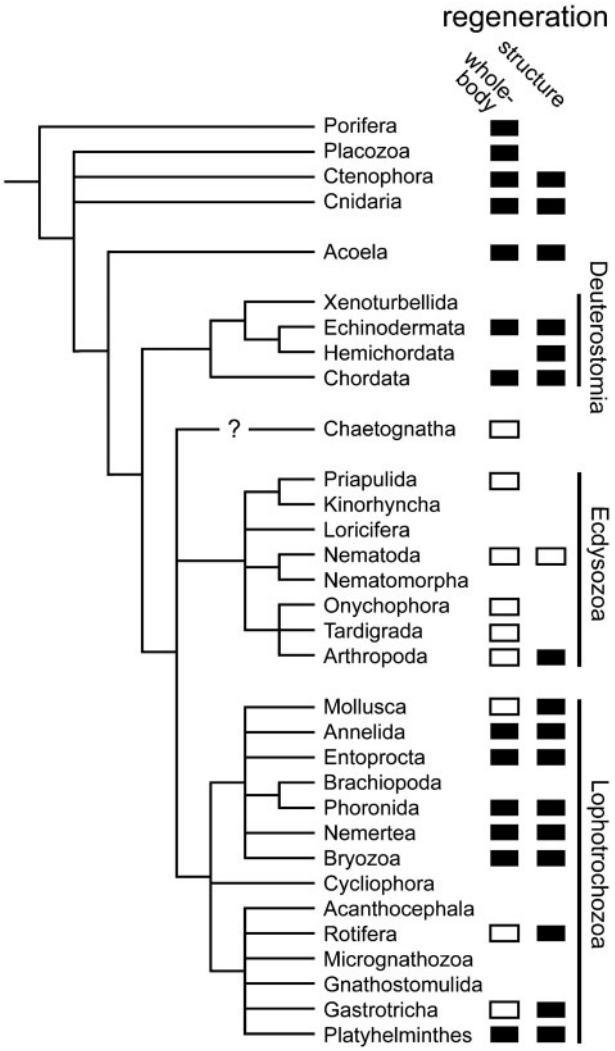

Fig. 1 Phylogenetic distribution of regenerative ability across the Metazoa. Regeneration is scored at two levels of organization: whole-body and structure-level regeneration. Whole-body regeneration is defined as the ability to regenerate every part of the body, although not necessarily simultaneously. Structure-level regeneration is defined as the ability to regenerate a multicellular structure of the organism (e.g., a head, tail, limb, or siphon) and excludes regeneration of internal organs or tissues. Black indicates that regenerative abilities are present in the phylum (regardless of whether non-regenerating species are also known from the phylum). White indicates that there is at least one substantiated report for the lack of regeneration in the phylum, and no evidence for the presence of regeneration. "?" indicates uncertainty in the phylogenetic position of this lineage. Modified from Bely and Nyberg (2010).

Second, even for groups for which a considerable amount of published information is available, there has undoubtedly been a strong publication bias against reporting failure of regeneration. The absence of regeneration is often interpreted as inconclusive, uninteresting, or simply not worth publishing. As an example, it is widely known that the nematode model organism *Caenorhabditis elegans* cannot regenerate at the "structure" level, such as after a transverse body amputation (although this species is capable of axonal regeneration; Yanik et al. 2004). Indeed, this species, and possibly nematodes in general, appear unable to wound-heal or even to survive body amputation because high internal pressure causes internal organs to be propelled from the body following a breach of the body wall. I could find no published documentation for this inability to survive body amputation or to regenerate, however, despite the fact that *C. elegans* is one of the most intensively studied animals in biology. The bias against publishing failure of regeneration has likely been pervasive, and this should be considered carefully whenever information on regenerative ability is gleaned solely from the published literature. Ignoring this potential bias may lead to the false impression that certain groups (e.g., planaria, echinoderms, ascidians) are uniformly good regenerators.

Third, reconstructing evolutionary changes in regenerative ability has been hampered by the lack of formal mapping onto robust phylogenies in some groups. Even if good information is available on the regenerative ability of many species in a group, without an estimate of their relationships it is simply not possible to reconstruct evolutionary changes in regenerative ability. The fact that regenerative ability appears common in a group does not imply that it is necessarily ancestral; if the most deeply branching lineages are all non-regenerating, or the developmental basis of regeneration differs in fundamental ways among lineages, a gain of regeneration may be a more likely reconstruction (e.g., Garza-Garcia et al. 2010). One group in which considerable information is available but no formal mapping has been performed is spiders. Many species can regenerate their legs and many species cannot (Vollrath 1990). Until these data are mapped onto a robust phylogeny, viewing non-regenerating species as representing losses should be considered tentative at best. A related problem inherent in character mapping is the necessity to make assumptions about the relative probability of gains versus losses. There is extremely limited knowledge of this for regeneration, leading different authors to sometimes make opposite conclusions about the polarity of change, based on the same information (Brockes et al. 2001). Even if reasonable estimates of the probabilities of transition can be obtained, these are likely to be taxon-specific and not easily applied more broadly.

Finally, the problem of homology is significant when different species exhibit high morphological disparity. Comparing regenerative potential across

different animal phyla, for instance, is inherently problematic because homologizing body parts is difficult at best, and can be downright nonsensical. For example, regeneration of sea star arms is a classic example of regeneration in deuterostomes, yet it is not possible to homologize sea star arms in any simple way to structures in other deuterostomes, such as hemichordates or chordates. Unlike the first three challenges listed above, which can be addressed relatively easily through targeted efforts, the ''homology problem'' is not easily overcome. Comparisons of regeneration across highly divergent taxa may need to focus specifically upon gains and losses of homologous developmental processes, rather than upon gains and losses of the ability to regenerate homologous morphological structures.

It should be noted that the cellular and molecular basis of regeneration can serve as an important source of evidence supporting or refuting the hypothesis of homology of regeneration across taxa, bearing directly on how gains and losses of regeneration are reconstructed. Obtaining this kind of information requires sustained, targeted efforts and a considerable financial investment, and it is thus currently not possible to use this approach across large comparative datasets. As the process of regeneration becomes increasingly well understood in a few model organisms, however, comparing findings across these models will become a key source of information for inferring how regenerative capabilities have evolved (e.g., Garza-Garcia et al. 2010). With the advent of molecular technologies that can be applied across a wide array of organisms (e.g., RNA interference, next-generation sequencing), it is becoming feasible to branch out from a model system to investigate the regenerative process in close relatives. Such studies will undoubtedly provide a rich source of information for evaluating where losses and gains of regeneration have occurred across animals.

Overview of regenerative abilities across the Metazoa

As discussed above, comparing regenerative ability across phyla is inherently problematic because body plans are so divergent that it is difficult to compare the ability to regenerate specific, homologous body parts. Even so, evaluating broad patterns in regenerative ability across the Metazoa is useful background for identifying losses of regeneration. Even a cursory survey of regenerative ability reveals marked variation among animals (Vorontsova and Liosner 1960; Goss 1969; Bely and Nyberg 2010). At one extreme, some groups such as sea stars, planaria, sponges, and hydrozoans have representatives capable of regenerating a complete individual from just a tiny fragment of the original, while at the other extreme, groups such as nematodes, leeches, and birds appear to be nearly or entirely unable to regenerate any structure. Are there broad patterns to this variation?

Among animals, all of the outgroup lineages to the protostomes and deuterostomes include powerful regenerators; sponges, cnidarians, placozoans, ctenophores, and acoels each have representatives that can regenerate following a range of amputations, even severe ones, and all have representatives with the potential to regenerate every structure in the body (Fig. 1). These groups also have few or no known species incapable of regeneration. Given this information, it seems likely that the common ancestor of animals, and early animals in general, were good regenerators. Consistent with this conclusion, many simple, soft-bodied animals today have high regenerative abilities. The alternative scenario, that most or all of these animal lineages acquired regenerative abilities independently, seems unlikely, although this possibility cannot be formally excluded based on available data.

Within the main bilaterian clade, comprised of the deuterostomes, ecdysozoans, and lophotrochozoans, regenerative abilities are, by contrast, highly variable (Fig. 1) (Needham 1952; Vorontsova and Liosner 1960; Goss 1969; Bely and Nyberg 2010). While regeneration of structures occurs in all three of these bilaterian clades (e.g., regeneration of siphons of ascidians, limbs of arthropods, heads and tails of planarians), many taxa in all three clades are capable of only limited regeneration, or of no regeneration at all (e.g., birds, nematodes, leeches). Assuming the ancestral animal could regenerate well, these non-regenerating bilaterians must represent losses of regeneration at some level, but determining when losses occurred relative to their closest regenerating relatives is not necessarily straightforward. This is because, although this phenomenon remains poorly documented, regenerative abilities can surely increase as well as decrease, and the pattern and timing of gains and losses can therefore be difficult to evaluate. The distribution of regeneration of tails across major groups of vertebrates illustrates this point. The tails of fish, amphibians, mammals, reptiles, and birds are homologous, but the ability to regenerate this body region varies considerably among them. Available evidence suggests that cartilaginous and ray-finned fish cannot regenerate their tails (the tail fin of some fish can regenerate, but not the bony elements), some amphibians can, mammals cannot, many lizards can, and birds cannot (Needham 1952; Vorontsova and Liosner 1960; Goss 1969). The evolutionary

pattern of regeneration of tails is clearly not simple even within vertebrates, as it must involve multiple gains and/or multiple losses. In vertebrates, as in many other bilaterian groups in which regenerative ability is variable, confidently reconstructing the pattern of the evolution of regeneration will require additional sampling, rigorous phylogenetic mapping, and optimally, data on the developmental basis of regeneration.

Some likely losses of regeneration

In some animal groups, the case for loss of regeneration is quite strong. One of the clearest examples comes from a recent study on annelids indicating that multiple losses of the ability to regenerate the head have occurred within a subfamily of aquatic clitellates, the Naidinae (Bely and Sikes 2010; see penultimate section). Comparative experiments on regenerative ability were performed on a range of species and, in each, the species-specific number of "head" segments was removed, such that amputations represented a comparable challenge across species. Mapping results onto a molecular phylogeny indicated that three losses of the ability to regenerate the head have occurred in this group.

Although this study represents the first to explicitly map regenerative ability onto a phylogeny as a means of reconstructing the pattern of losses, data from several other groups suggest additional likely losses when interpreted in the context of current understanding of phylogenetic relationships. For example, in lepidosaur vertebrates (lizards and close relatives), the ability to regenerate the tail is widespread, having been reported in the tuatara (the sister group to the rest of the lepidosaurs), geckos, skinks, iguanids, some agamids, chameleons, and lacertids, but it appears to be absent in a few groups, including snakes, amphisbaenids, and some agamids (Gans 1978; Arnold 1984; Bellairs and Bryant 1985; Seligmann et al. 2008). Based on a recent phylogeny of the group (Vidal and Hedges 2005), this distribution strongly suggests that tail regeneration is ancestral for lepidosaurs and that the non-regenerating groups likely represent at least three independent losses of the ability to regenerate the tail. Further support for tail regeneration being ancestral (and thus for the lack of tail regeneration being derived) comes from the regeneration process itself. When regeneration of the tail occurs in lepidosaurs, it consistently produces a regenerated tail that is distinctly different from the original; most notably, the regenerated tail has a continuous, cartilaginous rod at its center rather than an articulated column composed of vertebrae (Etheridge 1959; Arnold 1984; Bellairs and Bryant 1985; Seligmann et al. 2008).

The distribution of regeneration of fins in fish provides another likely example of regeneration loss. Fin regeneration occurs in lungfish (an outgroup to the teleosts), salmoniforms and esociforms (which together likely represent the sister group to the spiny-rayed teleosts), and numerous species of spiny-rayed teleosts (the most diverse group of living teleosts), including the cyprinodontiforms, atheriniforms, and perciforms (Wagner and Misof 1992 and references therein). However, regeneration of fins appears to be absent or heteromorphic (producing a misshapen, abnormal fin) in representatives of at least three groups of spiny-rayed teleosts: the scorpaeniforms, the cyprinodontiforms, and the perciforms (Wagner and Misof 1992). In the context of a recent summary of fish phylogeny (Nelson 2006), this distribution would suggest that regeneration of fins is ancestral for fish and that there have been multiple losses of normal fin regeneration.

A number of additional animal groups are known to possess a mix of species (sometimes even very closely related species) that can and cannot regenerate a specific body region, a pattern suggestive of loss of regeneration. Such groups include arachnids, hexapods, annelids, platyhelminths, nemerteans, hemichordates, urochordates, and amphibians, among others (Needham 1952; Kolmayer and Stéphan-Dubois 1960; Vorontsova and Liosner 1960; Stephan-Dubois and Bautz 1975; Scadding 1977, 1981; Vollrath 1990; Tsonis 2000; Brockes et al. 2001; Bely 2006). Although it is likely that many of the non-regenerating taxa represent losses of regeneration, better sampling and formal phylogenetic mapping are needed in most cases to provide evidence for this.

Finally, although comparisons of regeneration among phyla are inherently problematic, owing to the extreme morphological disparity among groups, a broad, phylum-level view of animal regeneration nevertheless suggests that an important restriction in regenerative ability occurred near the base of the Ecdysozoa, the molting clade of bilaterians. While non-bilaterians, acoels, deuterostomes, and lophotrochozoans each possess many representatives capable of "whole-body" regeneration (defined as being able to regenerate every part of the body), no such abilities are known from ecdysozoans (Fig. 1). Furthermore, while there is ample evidence that a broad range of body structures (e.g., heads, tails, limbs, siphons) can potentially regenerate in the other two main bilaterian clades (the deuterostomes

and lophotrochozoans), the only structure-level regeneration known from ecdysozoans is the regeneration of arthropods' appendages (limbs and antennae) (Vorontsova and Liosner 1960; Goss 1969). Regeneration of appendages occurs in many, although by no means all, arthropods, and because regeneration in arthropods depends on molting, no regeneration can occur in individuals after their terminal molt. The inferred restriction of regenerative ability near the base of the Ecdysozoa likely represents the oldest major loss of regeneration represented among metazoans, and may have been related to the evolution of the protective cuticle characteristic of this clade.

The process of loss of regeneration

Very little work has been aimed at understanding the process of regeneration loss, and we therefore know little about why and how this process occurs. For well over a century there has been speculation about the factors leading to loss of regeneration (Morgan 1901; Dinsmore 1991; Goss 1992; Wagner and Misof 1992; Bely and Nyberg 2010), but there remains a real need to define and evaluate specific evolutionary and developmental scenarios that could account for this phenomenon. To begin developing a framework for investigating this process, below I discuss some possible scenarios for both why and how regeneration could be lost. It will be important to evaluate such scenarios using actual data, ideally by investigating very recent losses in which the signatures of regeneration loss are most likely to still be detectable.

Why might regeneration be lost?

With regard to the ultimate, or evolutionary, causes of regeneration loss, a range of possible explanations have recently been outlined and discussed (Bely and Nyberg 2010). In summary, regeneration could be lost either because it is selected against in some way, or because it is a neutral trait. Regarding the first, selection could directly disfavor regeneration because it somehow contributes to lower fitness, as could occur if a partially regenerated structure impairs an organism's function more than the total absence of the structure (Vollrath 1990). Regeneration could also be disfavored indirectly, for example if there is an energetic trade-off between regeneration and another process, such as growth (e.g., Lawrence 2010; Wulff 2010) and investment in the latter becomes favored at the expense of the former. Adaptive explanations need not be invoked for loss of regeneration, however. If regeneration confers no significant selective advantage, then it could be lost as a neutral trait. A straightforward scenario under which regeneration might be neutral is if the structure in question is infrequently lost or damaged in nature. There is ample evidence that even traits recently under strong selection can be rapidly lost or modified when selection in the wild becomes relaxed (Lahti et al. 2009). Sublethal predation is a prevalent cause of regeneration in nature (Lindsay 2010), and thus this is presumably a common selective force maintaining regeneration. A simple change in predator-prey dynamics that decreases the frequency of sublethal predation in a population could therefore lead to regeneration no longer being actively maintained. Regeneration could also be neutral if the functional importance of the structure in question is so high that the animal cannot survive without it long enough to regenerate it, or so low that the structure is not worth replacing given the costs of replacement (Goss 1969; Reichman 1984). Neutral loss of regeneration could also occur if previously tight developmental pleiotropies between regeneration and another process (e.g., embryogenesis) simply break down, such that redeployment of the latter's developmental program is no longer possible in the adult stage (Galis et al. 2003). This scenario could explain decreases in regenerative ability that correlate with changes in morphology or changes in the developmental processes underlying the morphology.

Distinguishing between these possible ultimate explanations for loss of regeneration is no small task. Doing so will require collecting and integrating functional, ecological, and developmental data for both regenerating and non-regenerating species, preferably closely related ones. Only by tackling this problem with hypothesis testing and actual data, however, can we begin moving away from the pervasive speculation that has thus far characterized discussions about the ultimate causes of regeneration loss.

How might regeneration be lost?

Uncovering the proximate causes of regeneration loss involves deciphering which steps of regeneration have become abrogated. Some of the broader questions we should be aiming to address with such data include: Does regeneration fail in different ways in different groups of animals? Are certain steps of regeneration particularly prone to becoming blocked? Does loss of regeneration occur all at once or gradually, and, if the latter, what do intermediate stages of the process look like?

The first step towards addressing these questions is to determine when and how the regenerative process

halts or becomes abnormal. Does wound-healing fail? Does a blastema (the mass of undifferentiated cells from which new structures form) not develop? Is the blastema improperly patterned? Is the regenerated structure missing certain functionally important elements? Although data are still limited, it is clear that failure to regenerate can manifest itself in a variety of ways and, interestingly, that there may be phylogenetic trends in how regeneration tends to fail. For example, when regeneration of fish fins fails, a structure often regenerates but is heteromorphic and misshapen (Wagner and Misof 1992); with amphibian limbs, a blastema often forms but is poorly patterned, such that no obvious structures are formed (Scadding 1977, 1981); with annelids' heads or tails, animals typically wound-heal but fail even to develop a blastema (Bely 2006; Bely and Sikes 2010); and with spider legs, wound-healing itself often fails if the amputation is positioned somewhere other than at a limb joint, such that animals leak body fluid and die from the amputation, never properly wound-healing (Vollrath 1990). Thus, available evidence suggests that regeneration may fail in predictable ways within a taxonomic group. This could occur if, within a given lineage, certain steps of the regenerative process are particularly prone to becoming blocked.

Although loss of regeneration theoretically could occur by the evolution of a single mutation that completely abrogates regeneration, it is also possible that regeneration is lost gradually, with populations at successively more advanced stages of regeneration loss showing increasingly restricted or poorer regenerative abilities. Specific hypotheses for how such gradual losses might occur need to be developed. It is already known that speed or success of regeneration can be sensitive to a variety of factors, such as the

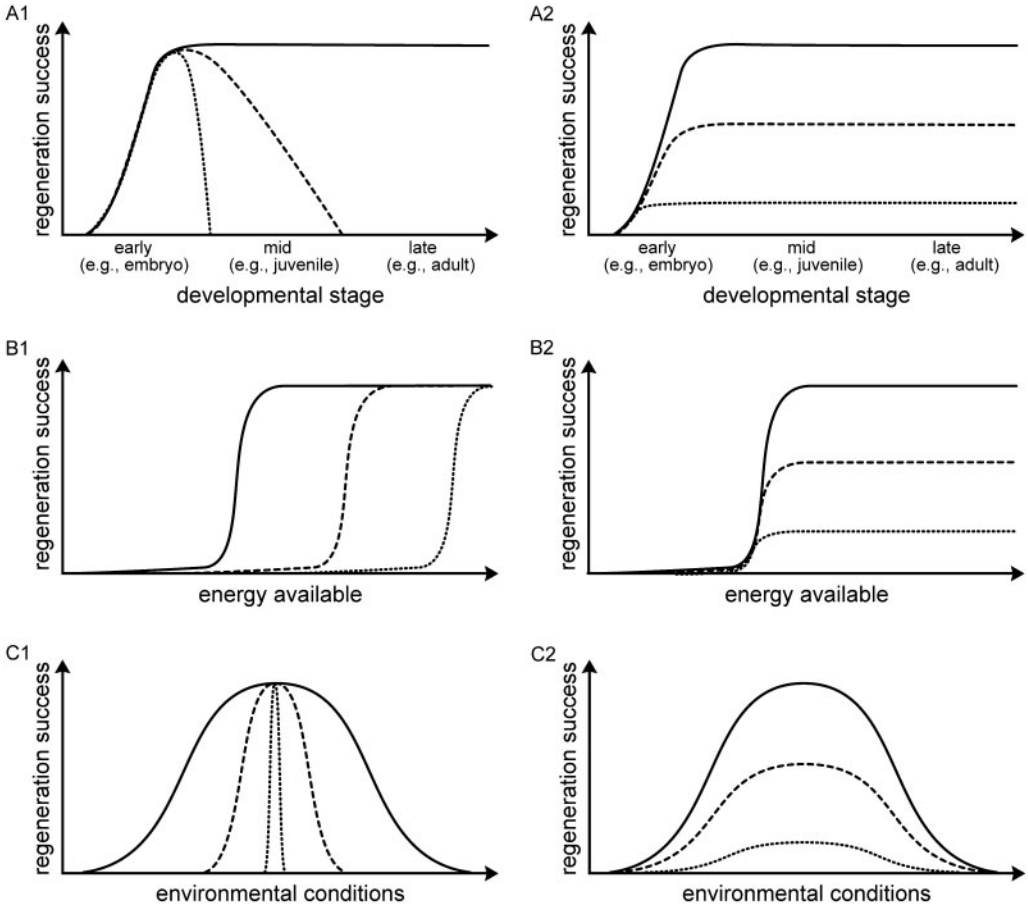

Fig. 2 Some hypothetical models for the gradual loss of regenerative abilities in a lineage. "Regeneration success" could be measured a number of ways, such as the frequency of successful regeneration, the morphological fidelity of the regenerated structure, or the functionality of the regenerated structure. Graphs on the left (A1, B1, and C1) show scenarios in which loss of regeneration is sensitive to a particular factor (developmental stage, energy available in an organism, and environmental conditions); graphs on the right (A2, B2, and C2) show scenarios in which loss of regeneration is insensitive to these factors. Solid lines denote the ancestral condition; dashed and dotted lines denote populations progressively farther along on the trajectory to total loss of regeneration. Curves for populations that have completely lost regenerative abilities would lie along the *x*-axis.

developmental age of the stump tissue and the energetic resources of the individual (e.g., Muller et al. 1999; Han et al. 2008; Lawrence 2010). In populations that are in the process of losing regenerative abilities, the success of regeneration could be contingent upon such factors as well, and, if so, identifying the specific factors that are still permissive of regeneration may point to an underlying mechanism for the loss.

Figure 2 shows several possible scenarios for how regeneration success could change through time in a lineage that is gradually losing regenerative abilities. One possibility is that successful regeneration becomes increasingly restricted across the life cycle. For example, regeneration could become restricted to earlier ontogenetic stages (Fig. 2A1), perhaps because younger tissues are comprised of cells with greater developmental flexibility. Another possibility is that the energetic threshold for investment in regeneration gradually increases (Fig. 2B1). Energetic tradeoffs between regeneration and other process, such as growth, are common (Maginnis 2006; Lawrence 2010), and if investment in other processes becomes favored at the expense of regeneration, the energy threshold for investment into regeneration could increase to the point that investment in regeneration effectively never occurs. Yet another possibility is that the environmental conditions permissive of regeneration could become increasingly narrow (Fig. 2C1). For example, regeneration might only occur within an increasingly narrow range of temperature or salinity. Other models for a gradual loss of regeneration may involve less easily quantifiable factors. For instance, success in regeneration may correlate with the degree of pleiotropy between regeneration and a core process, such as embryogenesis. If regeneration is being lost because this pleiotropy is breaking down, the fidelity of regenerated structures might gradually decrease through time. Models such as the ones shown in Fig. 2 provide clear predictions about how success of regeneration might differ in lineages at different stages of regeneration loss. To test the validity of such models, the contingency of regeneration success on internal and external factors should be evaluated in taxa that have lost or appear to be losing regenerative abilities, as well as in closely related, fully-regenerating taxa which can serve as a proxy for the ancestral, fully-regenerating population.

The holy grail for understanding how regeneration has been lost is to identify the actual genetic change(s) responsible for the original failure of regeneration. Although this is an important goal, it is essential to recognize that once a block to normal regeneration has evolved in a lineage, rendering regeneration non-functional, additional blocks may rapidly accumulate. Thus, in all but the most recently evolved cases of regeneration loss, regeneration is likely to fail for multiple reasons, and pinpointing the first block(s) responsible for the abrogation of regeneration will be challenging at best. Currently no species are known that show natural, intraspecific variation in success of regeneration, but if such species can be identified, they will be extremely useful for elucidating the earliest steps of loss of regeneration at the molecular level.

Annelids as a model for studying loss of regeneration

Annelids exhibit extensive variation in regenerative ability, making them useful models for understanding regeneration loss. The ability to regenerate a new tail is widespread across the phylum, having been lost in only a few taxa (Bely 2006). The ability to regenerate the head is much more variable, however, suggesting numerous losses. Evidence for regeneration of anterior segments was recently compiled for the phylum (Bely 2006). By mapping this information onto a molecular phylogeny for the group from Struck et al. (2007), I show here that annelids incapable of regenerating anterior segments are scattered across the phylum (Fig. 3A). There is still considerable uncertainty about the deep-level phylogeny of annelids (Rousset et al. 2007; Struck et al. 2007; Zrzavý et al. 2009) and greater sampling of regenerative ability is clearly needed, but the available data are consistent with the ability to regenerate anterior segments being ancestral for the phylum and subsequently being lost many times. Some groups, most notably the Hirudinida and Nereididae, are large clades thought to be comprised entirely of species that cannot regenerate anterior segments; these taxa likely represent relatively old losses of the ability to regenerate anteriorly. However, seven annelid families (representing over one quarter of the families for which there are relevant data) possess both anteriorly regenerating and anteriorly non-regenerating species (Fig. 3A, split black/white circles), suggesting at least this many recent losses. As sampling for regenerative ability is increased, the number of suggested losses is likely to increase as well. Groups in which regeneration has been lost relatively recently are particularly useful for investigating the mechanisms underlying the loss of regeneration.

To identify recent regeneration losses rigorously, fine-scale sampling and detailed phylogenies must be obtained. Such work has recently been carried out within the family Naididae (former Tubificidae [Erséus et al. 2008]), focusing specifically on the

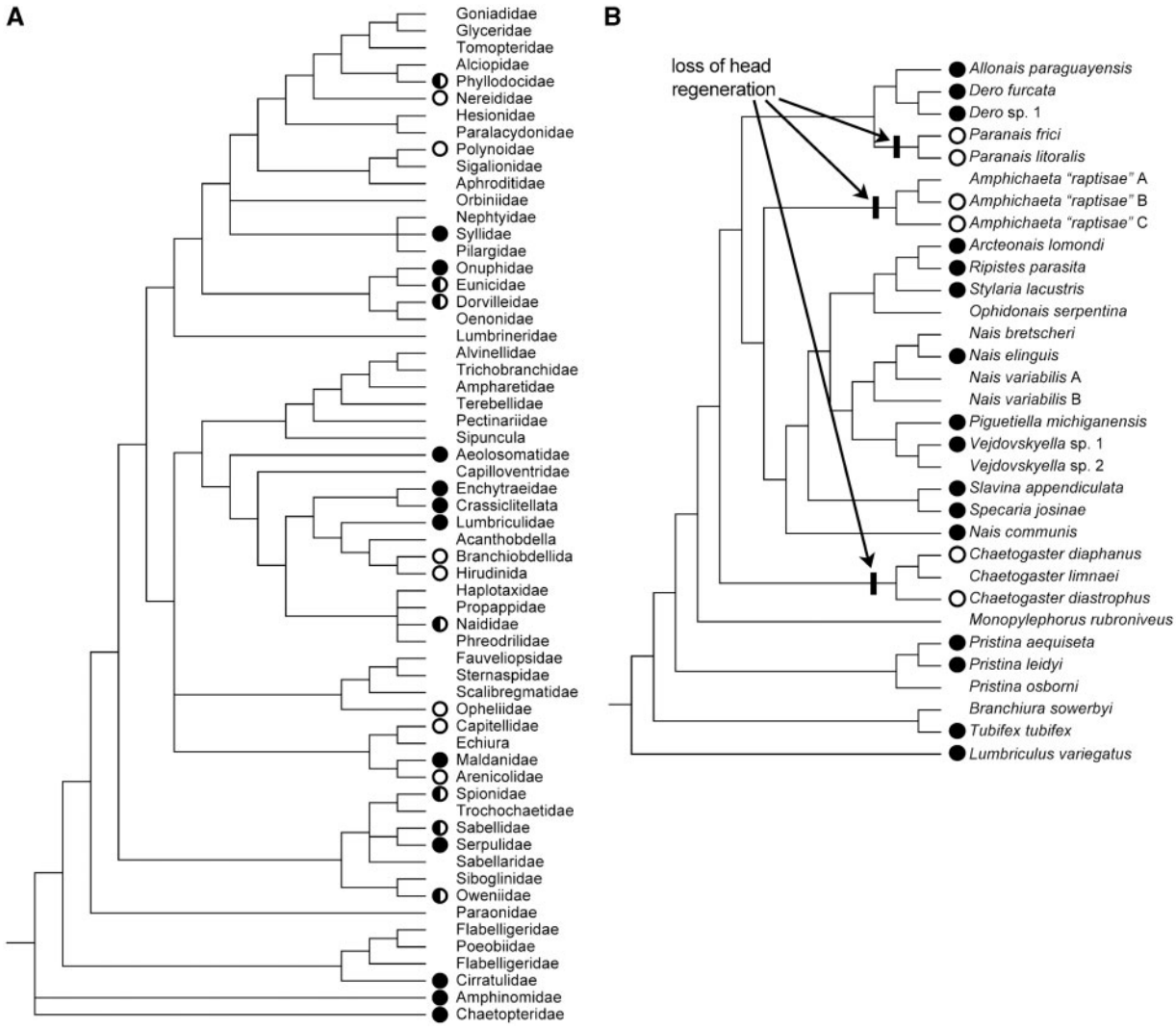

Fig. 3 Phylogenetic distribution of anterior regeneration in the annelids across (A) the entire phylum and (B) the clitellate subfamily Naidinae. Black circles denote groups in which all taxa investigated have been shown to have the ability to regenerate anterior segments; white circles denote groups in which all investigated taxa lack the ability to regenerate anterior segments; split black/white circles denote groups in which some species have been shown to regenerate anterior segments while others have been shown to be incapable of doing so. Data on regeneration are based on (A) Bely (2006) and (B) Bely and Sikes (2010). Phylogenetic relationships are based on (A) Struck et al. (2007) and Erséus and Källersjö (2004) and (B) Bely and Sikes (2010). In (A), all taxa are annelids, and most, although not all, groups represented are families. In (B), all species shown are naidines with the exception of the outgroups *Monopylephorus*, *Pristina*, *Branchiura*, *Tubifex*, and *Lumbriculus*.

subfamily Naidinae (Bely and Sikes 2010). Naidines are a group of small freshwater oligochaetes that reproduce asexually by fission, a process thought to have evolved from regeneration (Bely and Wray 2001). Because several naidine species possess excellent abilities to regenerate both the anterior and posterior ends, and because naidines are all capable of asexual reproduction, it had long been assumed that this entire group consisted of excellent regenerators. However, Bely (1999) demonstrated that at least one naidine species, *Paranais litoralis*, could not regenerate anterior segments, even though it could regenerate posterior segments. Following up on this study, Bely and Sikes (2010) sampled more widely within the naidines and found that six of the 18 naidine species investigated are incapable of regenerating the head segments. Mapping these comparative data onto a robust, five-gene molecular phylogeny of the group indicates that these six species represent three independent losses of the ability to regenerate the head region, with the genera *Paranais*, *Chaetogaster*, and *Amphichaeta* each representing a loss (Fig. 3B). It should be noted, however, that other phylogenetic studies of

naidines, based on smaller datasets, indicate the possibility of fewer losses (Bely and Wray 2004; Envall et al. 2006; Erséus et al. 2010). One of the species found incapable of regenerating anterior segments, *Chaetogaster diaphanus*, is a common and abundant species and one of the naidine subjects of an early, thorough description focusing on fission and, to a lesser extent, regeneration (Dehorne 1916). That no mention of this species' inability to regenerate anteriorly has surfaced until now likely speaks to the publication bias against reporting failure to regenerate.

The study by Bely and Sikes (2010) also found that regeneration fails at approximately the same point in the process in the non-anteriorly regenerating naidines, suggesting that some aspects of regeneration loss may be predictable within this group. After anterior amputation, these species wound-heal but fail to form a detectable blastema, although in one lineage post-amputation cell proliferation is still initiated. Interestingly, other non-regenerating annelids similarly fail at this same point: after wound-healing and before formation of the blastema (Bely 2006). This suggests that, at least in annelids, regeneration is either preferentially, or most easily, blocked about the time of blastema formation. This could occur, for example, if selection is acting to halt regeneration as early as possible (e.g., if optimal allocation of energy favors investment in another process, such as asexual reproduction), if initiation of regeneration is the most susceptible phase of regeneration (e.g., if this phase represents the largest mutational target), or if initiation of regeneration is the least pleiotropic phase of regeneration, such that a block at this phase does not impair other critical processes that are being maintained (e.g., wound-healing, embryogenesis, and growth). Determining the underlying cause for the repeated evolution of early-acting blocks to regeneration in annelids clearly warrants further investigation.

The study by Bely and Sikes (2010) provides a rare glimpse at an intermediate phase of the loss of regeneration. Specifically, the data are consistent with a model in which regeneration becomes gradually restricted to earlier ontogenetic stages, as diagrammed in Fig. 2A1. Because naidines (and some of their close relatives) typically reproduce asexually by paratomic fission, in which a new head and tail form in the middle of the body (Fig. 4A), amputations can be performed within young developing tissue, yet still within the context of an otherwise fully formed adult (Fig. 4B, right). Such amputations were performed on three species, specifically bisecting the developing head (leaving a stump comprised of young developing tissue), or, for a control, cutting immediately behind the developing head (leaving a stump of old adult tissue). Results showed that *Pristina leidyi*, a close naidine outgroup capable of regenerating anteriorly from adult tissue, can regenerate anteriorly from a stump comprised of young developing tissue. At a first approximation, then, this species matches the solid line of Fig. 2A1 and likely represents the ancestral, fully-regenerating condition. *Chaetogaster diaphanus*, which cannot regenerate anteriorly in the adult, was found to also lack the ability to regenerate anteriorly from young developing tissue. Thus, this species appears to have completely lost the ability to regenerate anteriorly, regardless of the developmental age of the stump, and would be represented on the graph in Fig. 2A1 by a flat line along the *x*-axis. However, *Paranais litoralis*, a naidine incapable of anterior regeneration from adult tissue, was found still to be capable of anterior regeneration when the stump was comprised of young developing tissue (Fig. 4B, lower right), suggesting an intermediate phase of regeneration loss as represented by the dotted or dashed lines of Fig. 2A1. Although it is not known whether *C. diaphanus* actually represents an older loss of regeneration than does *P. litoralis*, the fact that *C. diaphanus* has lower regenerative potential by several additional criteria (Bely and Sikes 2010) is consistent with this lineage being "farther along" on the trajectory to regeneration loss than is *P. litoralis*.

The study of naidines is the first to provide clear evidence for regeneration being lost in an uneven way across the life cycle. A few other taxa, most notably some vertebrates, are known to possess higher regenerative abilities during the embryonic or juvenile phases of the life cycle than in the adult phase (Vorontsova and Liosner 1960; Wallace 1981). For example, human children and mouse embryos are capable of regenerating digit tips, even though adults of these species are not (Illingworth 1974; Han et al. 2008). However, no extant mammals are known to regenerate digit tips as adults, and there is no evidence that the mammalian ancestor could do so either. Thus, interpreting the vertebrate pattern as a phase of regeneration loss is problematic at best. Instead, it may be that actively developing tissue is generally more permissive of regeneration, and that this may be manifest in lineages that are losing regeneration, gaining regeneration, or experiencing no change in regeneration. It should also be noted that early phases of the life cycle are not necessarily more regenerative, as exemplified by groups such as ascidians which may have extremely high regenerative

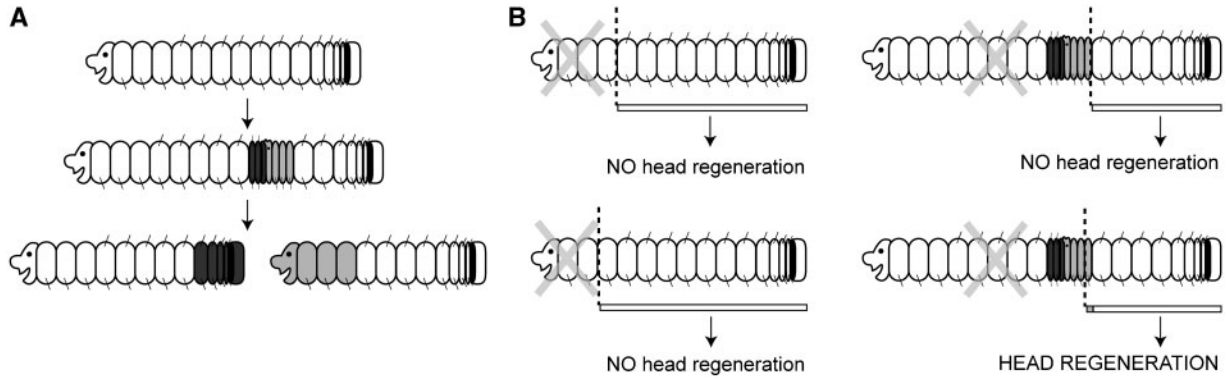

Fig. 4 Paratomic fission in naidines (A) and the ability to regenerate anteriorly in the naidine *Paranais litoralis* (B). (A) During paratomic fission, a new tail (dark gray) and a new head (light gray) are intercalated in the middle of the body of an adult worm, forming a transiently linked chain of individuals. Worms eventually separate and can reinitiate the process. In *P. litoralis* (diagrammed here), the new head made within the fission zone is comprised of three head segments, which are devoid of dorsal chaetae (bristles), and the asegmental cap of tissue bearing the mouth. (B) *P. litoralis* cannot regenerate if the amputation leaves a stump consisting entirely of old segments (white) (diagrams at top left, bottom left, and top right) but can regenerate if the amputation leaves even a small zone of actively developing tissue (light gray) at the distal end of the stump (diagram at bottom right). Diagrams on the left portray amputations in adult tissue: removal of all three head segments (top), two head segments (bottom), or even a single segment (not shown) result in wound-healing but not in regeneration. Diagrams on the right portray amputations adjacent to, or within, the fission zone: removal of all three head segments forming within the fission zone (top) results in wound-healing but not in regeneration, while removal of two head segments (bottom) or one head segment (not shown) from the fission zone can result in regeneration of a complete head. Results are from Bely and Sikes (2010).

abilities as adults yet none demonstrated during the larval phase (Vorontsova and Liosner 1960).

Why, ultimately, regeneration has been lost in several naidines is as of yet unclear. There is ample evidence that some annelids experience high levels of sublethal predation (De Vlas 1979a, 1979b; Clavier 1984; Berke et al. 2009; Lindsay 2010), and thus it is likely that regeneration is selectively favored in many annelid species. Sublethal predation has even been demonstrated in a naidine species (Kaliszewicz 2003; Kaliszewicz et al. 2005). However, there is no information on the incidence of sublethal predation for most naidines, including the six non-anteriorly regenerating species studied. Therefore, the hypothesis that regeneration loss in naidines occurred because of a decrease in the direct selective advantage for anterior regeneration remains to be evaluated.

The possibility that regeneration may have been lost through indirect negative selection should also be evaluated in the future, since there are likely energetic tradeoffs between the multiple post-embryonic developmental capabilities of naidines (tissue turnover, growth, fission, regeneration). An anteriorly amputated naidine can potentially invest in several different developmental processes, and if optimal allocation of energy favors investment in fission at the cost of regeneration, for example, regeneration could be indirectly selected against, possibly resulting in regeneration loss as diagrammed in Fig. 2B1. Indeed, when anteriorly amputated, *Paranais litoralis* is known to accelerate the fission process such that amputated individuals produce detached offspring more quickly than do unamputated individuals (Bely 1999). Whether regeneration is actually delayed or inhibited when a fission zone is present should be studied in both anteriorly regenerating and anteriorly non-regenerating species to further investigate this possibility.

Future directions

Because regenerative ability can be influenced by a wide range of factors, developing a good understanding of the mechanisms responsible for evolutionary loss of regeneration will require taking a broad, integrative approach. Developing animal models that are amenable to a range of investigations will be particularly important, as this will allow studies of the functional biology, ecology, evolutionary history, and developmental genetic basis of regeneration to be integrated for the purpose of testing alternative hypotheses about loss of regeneration. While research into regeneration has traditionally been carried out on a few, distantly related model organisms, understanding the ultimate and proximate causes of regeneration loss requires a shift in approach; comparative studies, focused on closely related regenerating and non-regenerating taxa, are critical for investigating why and how regenerative abilities are lost. Groups

in which regeneration has been lost multiple times among close relatives represent particularly attractive systems for study, as these ''natural replicates'' may reveal separate steps in the process of regeneration loss and suggest which aspects may be predictable.

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