

Regeneration: Rewarding, But Potentially Risky

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Some bilaterally symmetric animals, such as flatworms, annelids, and nemertean, are renowned for their outstanding regeneration capacity—even a fraction of the body can give rise to a complete new animal. However, not all species of these taxa can regenerate equally well—some cannot regenerate at all. If regeneration was purely beneficial, why cannot all of members of the flat, round, and ribbon worms regenerate? At that, why cannot all other bilaterians, including humans, regenerate as well? Regeneration capacity is an obvious advantage in accidental, predatory, and parasitic loss of body parts and is also closely intertwined with asexual reproduction strategies. Regeneration is suspected to play a role in life span extension or even rejuvenation. An answer for reduced or missing regeneration capacity in many species may be found in limitations of the body plan, high costs, and inherent dangers of regeneration. Defects in adults and juveniles are shown, and similarities between development and regeneration are pointed out. With a focus on some worms, but also highlighting comparisons with other animal taxa, putative reasons for a limited and an advanced regeneration capacity are discussed in this article. **Birth Defects Research (Part C) 84:257–264, 2008.** © 2008 Wiley-Liss, Inc.

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INTRODUCTION

The ability to regenerate is certainly a useful feature. It provides an insurance for accidental, parasitic, and predatory loss of body parts and can also be used for clonal (asexual) reproduction (Fig. 1). Especially, flatworms have been in the focus of regeneration research since Pallas (1774) discovered the profound regeneration capacity of some triclad flatworms. They were touted as being “almost immortal under the edge of the knife” (Dalyell, 1814), as the experimental animals were found to survive cut after cut, each piece regenerating a new, albeit smaller, animal. In a famous, but often incorrectly cited article, Morgan (1902) described a detailed regeneration experiment of a triclad flatworm

that was cut into many pieces. Drawing the schematic outlines of his experimental species on cardboard and cutting out the shape, he obtained a reference body mass by weighing this cardboard flatworm. Subsequently, he cut off and weighed small parts of this cardboard model, in sizes proportional to the pieces he amputated from the actual flatworms. He thereby determined that amputated fractions as small as 1/279 of the body were able to show signs of regeneration; however, these smallest pieces could not regenerate a complete animal, but died earlier (Morgan, 1902). Later, Montgomery and Coward (1974) estimated the minimal cell number needed for regeneration in a triclad as about 10,000 cells. The

bar was lowered after cell countings in the small flatworm *Macrostomum lignano*: a piece measuring about 300 μm in length and containing about 4000 cells, including about 160 stem cells, suffices to regenerate a complete adult organism—a record not only among flatworms but even among bilaterians. In the same species, freshly hatched juveniles require about 1500 cells (plus 50 remaining stem cells) in a piece measuring 100 μm in length for complete regeneration and development of all adult tissues, even gonads (Egger et al., 2006). Regeneration from even smaller tissues—although of embryonic origin—is known from parasitic flatworms. Here, a single cell of a tapeworm’s cysticercus was injected into the mouse host and was able to form a new cysticercus giving rise to a functional larva (Toledo et al., 1997).

PREDATORS AND ABIOTIC FACTORS

Regeneration capacity can be regarded as an adaptation to the loss of body parts. Trauma may occur due to predators or abiotic stimuli prevalent in environments that are exposed to considerable mechanical force, such as turbulent waters or the interstitial system. Species can employ different strategies to survive in challenging habitats, such as burrowing into the substrate, by protecting themselves with hard shells, or, especially for soft-bodied creatures, by

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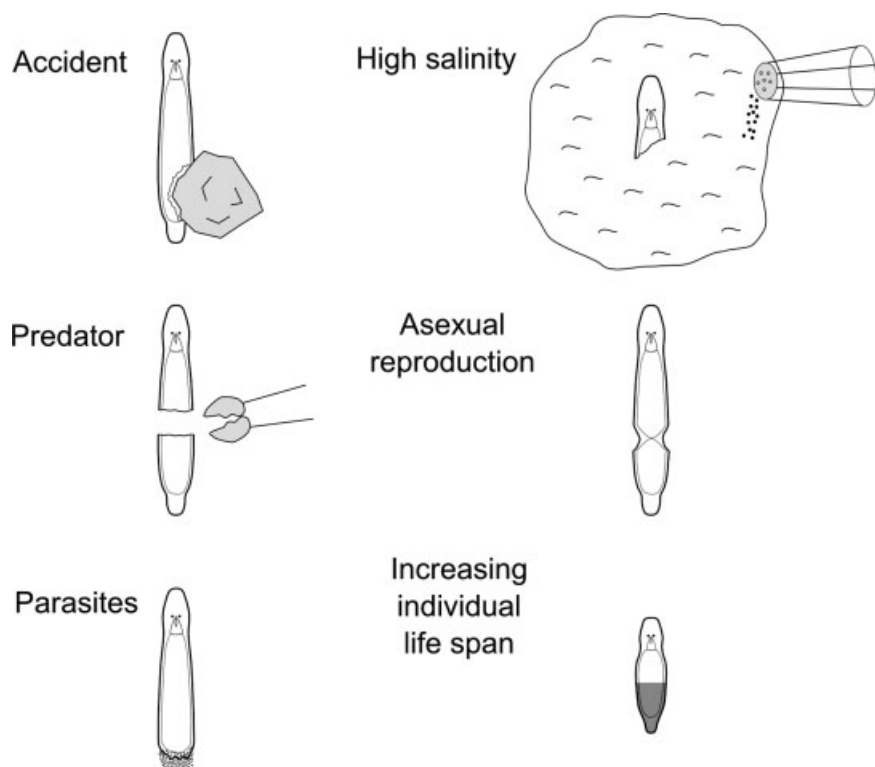


Figure 1. Illustration of scenarios where regeneration may be advantageous for an animal, exemplified by a flatworm. Wounds inflicted by abiotic stimuli, e.g., being crushed by a rock, or injury caused by predators or parasites can be dealt with animals capable of regeneration. Nonmechanical environmental influences, such as temporary hypersalinity conditions can also lead to partial loss of tissue and be restored by subsequent regeneration. Regeneration can also be part of an asexual regeneration strategy, and, probably as a side effect, increase not only clonal but also individual life span.

exhibiting a considerable regeneration ability. This is the case with many annelids (Fischer, 1999; Bely, 2006), flatworms (Saló and Baguñà, 2002; Egger et al., 2007), and some nemerteans (Bierne, 1990; Saló and Baguñà, 2002). Natural disasters, such as zoologists on a collecting trip, provide ample evidence for the existence of physical disruptions in the habitat; in many samples injured or regenerating animals can be found (personal observations).

Although a large number of chemicals has been tested on regenerating flatworms (e.g., Brøndsted, 1969), not much information is available on abiotic factors causing regeneration. Amputations with a knife incite the regeneration response, whereas irradiation and burns were not found to be able to trigger regen-

eration in freshwater triclads (Brøndsted, 1969). Animals treated in this manner will only start regeneration after the burnt or irradiated tissues have been at least partly amputated. An ubiquitous chemical, sodium chloride, can cause the dissolution of tissues in hypersalinic concentrations (5.5% instead of 3.2%) even in marine flatworms; such animals can be rescued by placement in more favorable culture conditions and will readily regenerate lost body parts without necessitating further amputations (personal observations). These findings identify another natural cause for regeneration in flatworms and possibly other animals as well (Fig. 1).

A variety of organisms are known predators of crinoid echinoderms: fishes, crustaceans, and other echinoderms, constituting a

selective pressure for arm regeneration capacity in these animals (Baumiller and Gahn, 2004). Other studies about the role of predators on the regeneration capacity are sparse, e.g., De Vlas (1979) on polychaetes and their flatfish predators. But even if some animals are seemingly adapted to cope with loss of body parts to predators or abiotic forces in their habitat, it remains unclear whether this adaptation is the primary driving force for regeneration ability or just a useful side effect.

AUTOTOMY

The most convincing evidence for the utilization of regeneration ability to cope with predation is autotomy: the spontaneous breaking of nonessential body parts along a predefined plane is mainly due to predation pressure. This defense strategy is employed by diverse taxa and has probably evolved independently a couple of times (Fleming et al., 2007 in a comprehensive review). Some cnidarians are able to autotomize their tentacles (Bickell-Page and Mackie, 1991). Leg autotomy is widespread in arthropods, although not all species can completely regenerate autotomized legs. Occurrence of arm autotomy and subsequent regeneration is widespread in echinoderms (Baumiller and Gahn, 2004), while some molluscs may sacrifice dorsal papillae or, like some annelids, their posterior part (Fleming et al., 2007).

Among vertebrates, lizards are best known for their ability to self-cut their tail as an escape strategy (Dial and Fitzpatrick, 1984).

CONSEQUENCES OF LOSING BODY PARTS

Autotomy and subsequent regeneration come with a cost: a reduced fitness by impaired movement, a decreased capacity for respiration, digestion, and energy storage, a lowered social status, and the need to allocate resources necessary for replacing the lost body parts (Maginnis, 2006;

Fleming et al., 2007; Naya et al., 2007).

Flatworms and annelids amputated posterior of the head usually do not exhibit striking behavioral changes, and seemingly move as fast, and in the case of head pieces not encumbered by the posterior part of the body, even faster as nonamputated specimens (personal observations). Headless anterior regenerates, however, are often almost motionless or move considerably slower until the head is at least partly regenerated (Kato et al., 2004). This can be explained by the role of the brain as an integrating and control center for directed locomotion. However, headless pieces often provide evidence for a partial autonomy in that they can still move and sometimes even feed with severed main nerve cords (Koopowitz et al., 1976) or in the complete absence of the brain (Egger et al., 2006). Both regenerating and intact animals usually seek shelter in openings and crevices and often avoid exposure to light. Distinct behavioral changes are observed in tailless lizards to compensate for deficiencies; for example, the movement rate and the home range size are decreased (Cooper, 2003).

PARASITES

Although about three quarters of the described flatworms are parasites themselves (Rieger, 1996), flatworms can fall prey to parasites as well. The comparatively large-bodied polyclad flatworms are known to be hosts of gregarines in the gut for some time (Lang, 1884), and recently unicellular parasites for the small-bodied flatworms, *M. lignano* and *Isodiametra pulchra*, have been identified. In *M. lignano*, this thraustochytrid parasite was described to preferentially enter the host through the posterior tip, causing "tailless" phenotypes in culture dishes (Schärer et al., 2007). The flatworms are able to regenerate the tail plate in favorable culture conditions. Similar phenotypes missing the tail plate were already

described by von Graff (1882) in freshly sampled specimens of another *Macrostomum* species, *M. hystrix*. However, he held that the loss of the posterior part in these animals was caused by predating crustaceans.

LIFE SPAN EXTENSION AND POSSIBLE REJUVENATION

Another curious side effect of regeneration was encountered in some flatworms and annelids: regeneration seems to prolong the life of amputated animals (Haranghy and Balázs, 1964; Martínez, 1996; Egger et al., 2006), a similar effect as observed in starved animals (Child, 1914; Haranghy and Balázs, 1964).

Repeatedly regenerated animals of *M. lignano* were found to live substantially longer than untreated culture animals (Egger et al., 2006, Ladurner et al., 2008). Besides the loss of eyes (the head was never amputated), a number of specimens did not show serious defects when compared with younger animals after 50 and more amputations (Fig. 2A,B). Even animals displaying heteromorphoses, such as multiple tail plates (Fig. 2C-F), survived almost 60 consecutive amputations. Thus, regeneration seems to provide means for life span extension, or possible even rejuvenation of the amputated body parts, as they are actually renewed during regeneration.

In the (very few) obligatory asexually reproducing animals, i.e., animals that were never found to produce sexually, the proposed way of tissue rejuvenation by regeneration is obviously taking place. For example, the annelid, *Dorvillea bermudensis*, has reproduced exclusively asexually (by architomic fission and regeneration) since laboratory cultures were established 30 years ago (Åkesson and Rice, 1992).

REGENERATION AND ASEQUAL REPRODUCTION

Regeneration ability goes hand in hand with asexual reproduction.

Some nemerteans tear apart easily and are subsequently able to regenerate lost body parts with apparent ease (Coe, 1929), that it seems these animals turned a potential weakness of their bauplan, the fragility, into a strategy to both cope with unfavorable conditions (caused by abiotic factors or predators) and to maximize asexual reproduction success.

All known modes of asexual reproduction (architomy, paratomy, budding, and fragmentation) can be found in various flatworms (Brøndsted, 1969; Egger et al., 2007). Architomy signifies the fission of a posterior—usually postpharyngeal—region before any differentiation of anterior structures has taken place in the posterior part. In contrast, paratomy is a fission event where head structures are formed in the posterior piece before fission occurs. Therefore, this kind of asexual reproduction involves "pregeneration" rather than regeneration. This is an important distinction, as some species can only regenerate a head (including brain) in the presence of a head, but fail to do so when the head is amputated (Egger et al., 2006, 2007). Budding is similar to paratomy, but the soon to be separated pieces are not orientated in the same axis as the parent animal, but in angles up to 180°, establishing essentially an anterior-posterior axis opposite to the parent animal (Hendelberg and Åkesson, 1988, 1991). Lastly, fragmentation denotes spontaneous breakup of animals due to rough handling or unfavorable culture conditions (Brøndsted, 1969), and it is therefore similar to architomy, but animals do not break at preformed fission planes.

DOES ASEQUAL REPRODUCTION EQUAL REGENERATION CAPACITY?

Generally, species using asexual reproduction are able to regenerate very well, both anteriorly and posteriorly. This is not only true for flatworms, but for annelids as

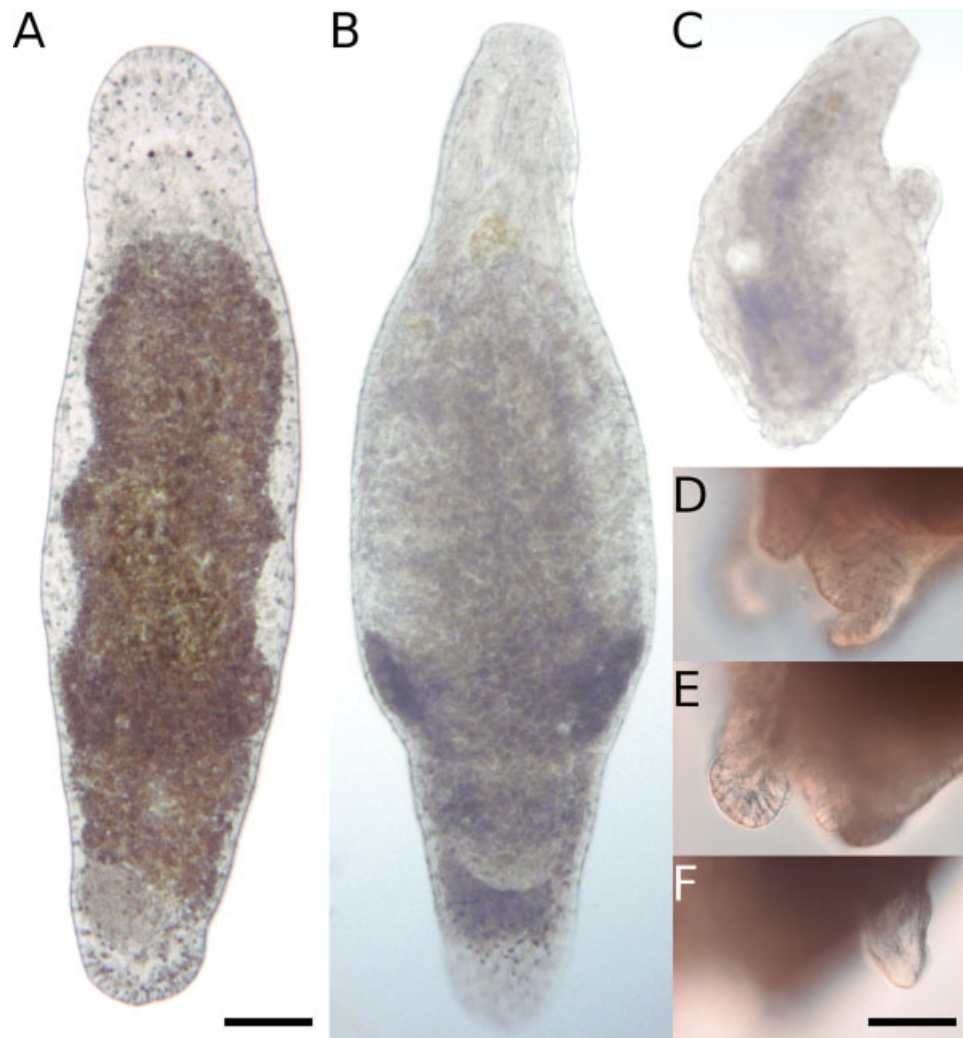


Figure 2. Repeated regeneration in *Macrostomum lignano* may lead to deformations in the axial patterning. Interference contrast images of live animals in squeeze preparations. **(A)** Nonamputated mature animal, about 3 weeks posthatching. **(B–F)** Animals after 50 consecutive amputations of posterior parts with subsequent regeneration carried out over a period of 22 months; note the lack of eyes in the head region, which were lost in all repeatedly regenerated animals about 10 months after the start of the experiment. **(B)** Besides the loss of eyes, no further deformations were detected in this specimen after 50 amputations. **(C)** Animal with hypertrophic testes and three tail plates after 50 amputations. **(D–F)** Focus and spatial series through the posterior part of an animal with seven tail plates after 13 amputations. Despite these deformations, the specimens shown in **(B–F)** survived the following amputations. Anterior is up. Scale bar for **(A–C)** is 100 μm , for **(D–F)** is 20 μm .

well, where also a clear correlation between anterior and posterior regeneration can be observed: species capable of regenerating anteriorly are also able to regenerate posteriorly. The opposite is not true; however, there is a number of species that can regenerate posteriorly, but not anteriorly (Bely, 2006). Again, similar findings are reported from flatworms (Egger et al., 2007).

There are exceptions, however, hinting that slightly different mechanisms are at work during asexual reproduction and during

regeneration. For example, in a catenulid flatworm that is reproducing by paratomy, artificial cuts were often unsuccessful in triggering a regeneration response (van Cleave, 1929).

Reuter and Kreshchenko (2004) have proposed that flatworm asexual multiplication implicates stem cells and regeneration. However, it is almost equally meaningful to turn it around: Regeneration implicates asexual reproduction. Generally, those animals that undergo asexual reproduction are also the ones that are

able to regenerate extremely well, both anteriorly and posteriorly (Bely, 2006; Egger et al., 2007). Thus, the ability to regenerate could be regarded as just a side effect, a by-product of asexual reproduction. This (monocausal) view of regeneration capacity as a secondary effect of the primary asexual reproduction goal is strengthened by the fact that in obligatory sexually reproducing animals the regeneration capacity is generally considerably diminished, with plenty of examples from flatworms, annelids, and

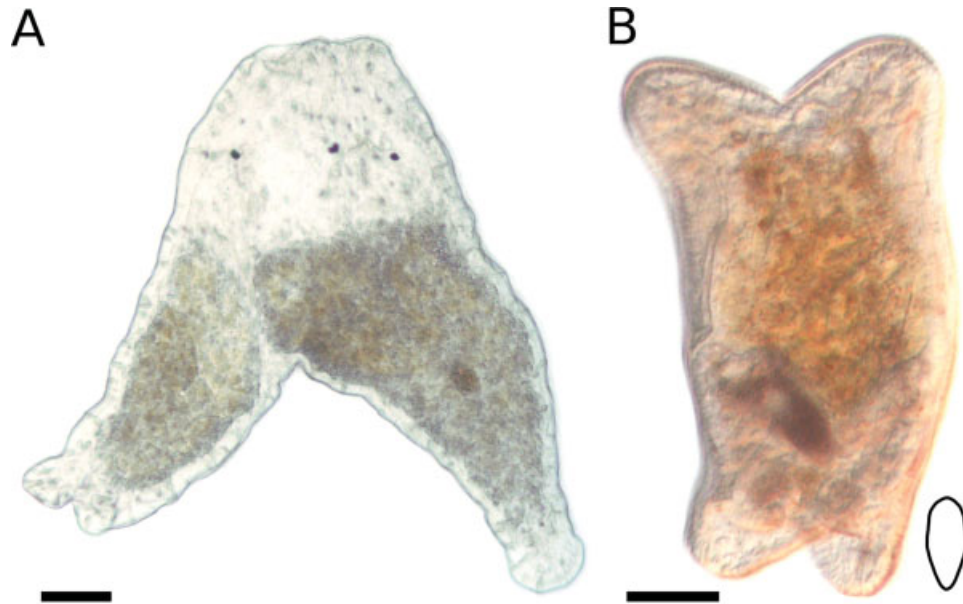


Figure 3. Heteromorphoses—misbuildings—by duplication of the body axes in the flatworms *Macrostomum lignano* (A) and *Isodiametra pulchra* (B). These defects likely originated during embryonic development, the animals have the size of young juveniles. (A) Partial duplication of the head, left head only has one eye. The animal on the left side also shows an additional bifurcation of the tail plate. The gut is separated by a narrow rim. (B) Complete duplication of the anterior-posterior axis with the formation of an additional head and tail region and a shared gut. Schematic drawing at the right bottom indicates the outline of a normal animal. Scale bars are 50 μm .

nemertean (Saló and Baguñà, 2002; Bely, 2006; Egger et al., 2007).

But why is the ability to regenerate so closely related to the needs of asexual reproduction? Why do we find so few animals that can regenerate very well, but do not undergo asexual reproduction? One answer is that it is essentially indistinguishable whether asexual reproduction leads to a formidable regenerative capacity, or whether this regeneration capacity also allows the animal to also employ asexual in addition to sexual reproduction strategies. If one is available, it is most sensible to also have the other.

Another answer is that regeneration comes with a cost: not only regarding resources and a (temporary) plunge in fitness as already discussed for autotomy, but in a more general way, regeneration may be dangerous.

THE DANGERS OF REGENERATION

Heteromorphoses are malformations manifested in regeneration

events (Fig. 2D–F) or during embryonic development (Fig. 3) (Egger et al., 2006, 2007). Flatworms are famous for their enormous capacity to be amendable for the most bizarre monstrosities: duplicated heads, tails, and whole body axes can almost be considered to be the mildest cases of deformities. By repeatedly incising animals in the head, 10-headed creatures can be experimentally obtained (Lus, 1924). By cutting a large hole (“window”) in flatworms, multiple supernumerary heads and tails in this hole can be obtained (Brøndsted, 1969). Even loops of anteriorly and posteriorly fused animals can be produced (Teshirogi and Watanabe, 1998).

Not only in experimentally induced animals but also in untreated culture dishes and during sampling trips, animals with strange aberrations can be found (Fig. 3) (Egger et al., 2007). This hints at an inherent risk that the ability to regenerate bears: that this ability is triggered not only in appropriate (emergency) situations, but also when it is not needed. Having two heads is not

an advantageous trait, flatworms with such malformations are usually degrading as a whole after a couple of weeks (Egger et al., 2006).

BODY PLAN RESTRICTIONS FOR REGENERATION

As seen earlier, many arthropods can regenerate—their legs. Why can they not regenerate the head or body segments? The answer is closely related to how they are able to regenerate appendages: they reappear at the molt only. Their tough exoskeleton effectively restricts the regeneration capacity, and also does not even allow closing large wounds easily, as the hemolymph would spill out and the animals die. At the end of the molting cycle, regeneration is no longer observed in arthropods (Bulliere and Bulliere, 1985). A similar argument can be made regarding nematodes: their hard cuticle protects effectively from outside dangers, but does seemingly not allow for proper wound closure, the first step necessary for successful regeneration (Sánchez Alvarado, 2000).

OTHER RESTRICTIONS FOR REGENERATION

Why can mammals, or most vertebrates at that, not regenerate very well? The head is never to be regenerated in this taxon, likely reflecting the importance of the brain for coordinating the body. But why not regenerate an arm, or a leg, or at least a finger? At least in human children and young mice, finger tips can sometimes be regenerated, but this ability is not present anymore in older children and adults (Han et al., 2008). Two factors may play a role here: the scar tissue formation preventing blastema formation, and possibly the reduced potency of the involved stem cell system. Scar tissue may be regarded as an effective way for keeping wounds closed and could be interpreted as being more advantageous in everyday life than an extensive regeneration capacity.

For regeneration to be a useful feature, a suitable environment is also necessary, allowing survival even after the loss of important, but nonessential body parts. As most animals capable of substantial regeneration are living in water bodies, this type of environment may be generally more favorable for surviving (often extended) regeneration periods.

Pronounced regeneration capacity in sponges and cnidarians suggests that regeneration capacity is an ancestral trait in bilaterians (Sánchez Alvarado, 2000) that was reduced or lost, and possibly reinvented several times. What was the selective pressure to lose a potential very useful feature? It appears that if regeneration capacity does not provide an immediate advantage for a given species, e.g., for asexual reproduction, it is often not considered a neutral feature, but is actively selected against (Goss, 1991).

STEM CELLS

Flatworms rely on their possibly totipotent stem cells, called neoblasts. These cells are the only dividing cells in adult flatworms and are responsible for growth,

tissue maintenance, and regeneration (Ladurner et al., 2008). They appear as small undifferentiated cells with a nucleus surrounded by a narrow cytoplasmic rim. Their morphology, together with their possible totipotency, has made scientists believe for now more than 100 years that these cells have retained an embryonic character (Keller, 1894).

REGENERATION, EMBRYONIC AND POSTEMBRYONIC DEVELOPMENT

At least in animals whose adults contain cells of "embryonic character" that are capable of regeneration (e.g., flatworms), it would be expected to find similarities between the embryonic development and regeneration. In triclad flatworm embryos, "striking similarities" have been identified in muscle and nerve development and regeneration (Cardona et al., 2005).

Because of the relative inaccessibility of the embryo, comparisons between the postembryonic development of developing juveniles and regeneration can be made more easily. Transparent models like many flatworms provide opportunities to study organ formation in vivo. For example, during regeneration of the tail plate in *M. lignano*, the similarities between regeneration of the tail plate and the postembryonic development of the tail plate are apparent. Day 3 of tail plate regeneration closely corresponds to day 8 of postembryonic development, when the tip of the stylet along with the vesicula granulorum are being formed (Fig. 4A,E). The complete differentiation of the stylet, along with the building of the seminal vesicles and the appearance of sperm in the vesicles, happens in a very similar time frame and pattern both during regeneration and postembryonic development (Fig. 4).

There are obvious differences between regeneration and postembryonic development: the tissues adjacent to the blastema in

adult regenerates are different (more mature) than in juveniles during the differentiation of, e.g., the male genital apparatus. Unique to regeneration are also the first two responses to amputation: wound healing and early blastema formation. After these first two steps specific to regeneration, it appears likely that both regeneration and postembryonic development invoke the same developmental pathways for organ differentiation in the tail plate. The co-option of existing pathways for different developmental processes seems to be an efficient way to adapt to new challenges (Sánchez Alvarado, 2000).

WHY CAN HUMANS NOT REGENERATE WELL?

The burning question remains! Why do humans not regenerate at least lost appendages? The simple answer is that it was not necessary for ensuring the survival of the species and may not even be beneficial without changing important parts of human physiology.

The more complex answer would likely address the specific response to injury involving scar tissue formation in mammals and the control of the stem cell system. Before being able to at least locally (e.g., at a finger stump) enhance the regeneration capacity, a thorough understanding of the stem cell system involved is necessary. Here, it becomes potentially dangerous—regeneration generally involves proliferating cells, and a large accumulation of undifferentiated proliferating cells, such as in a blastema, shares suspicious similarities with cancer cells.

Flatworms provide some evidence that a very potent stem cell system and the ability to regenerate is not always beneficial, although heteromorphoses and other developmental aberrations can also be found in species with no pronounced regeneration capacity. One difference is that in flatworms, such deformations can also occur during postembryonic development and in adults, while in many other animals the capacity to

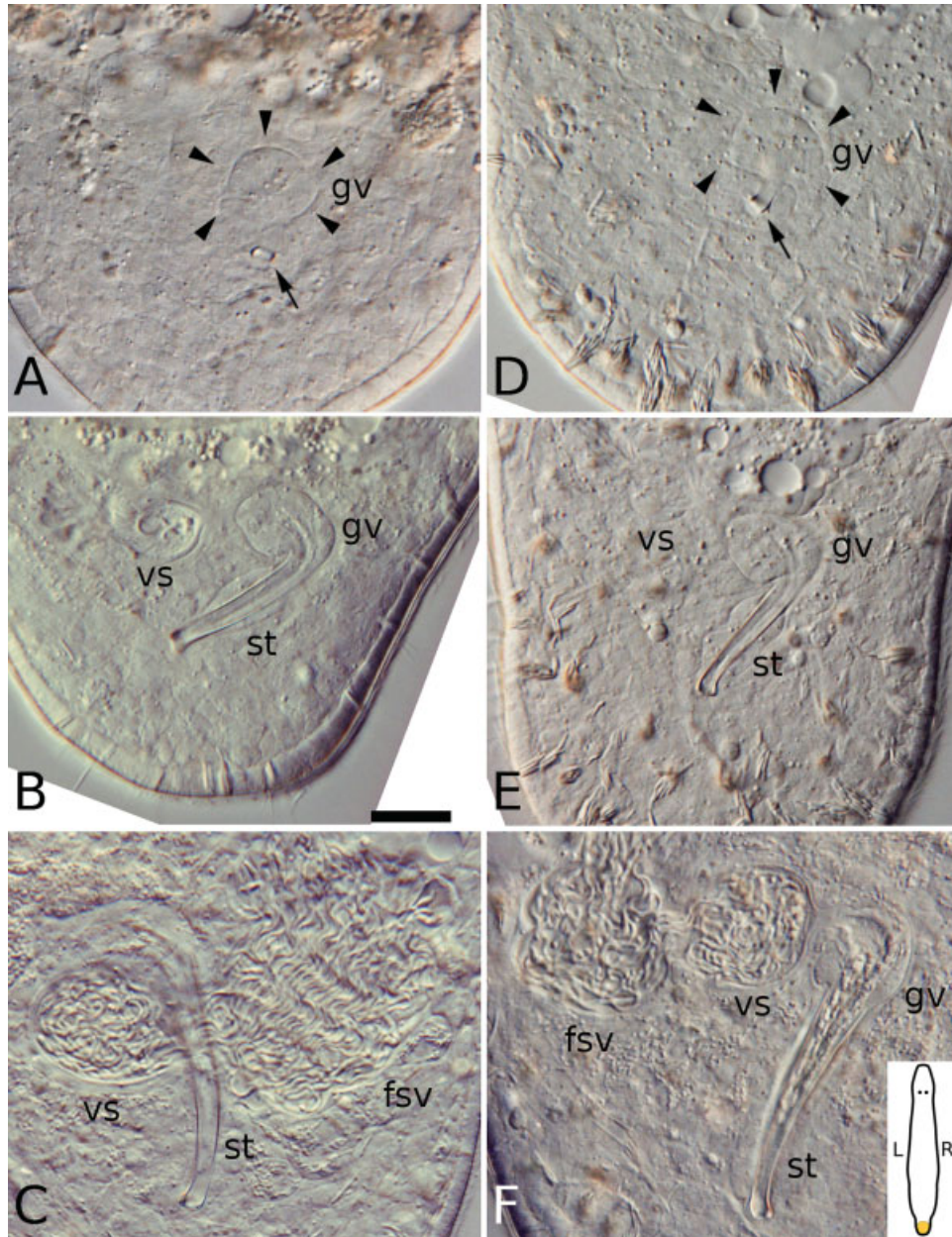


Figure 4. Similar events are found during regeneration and postembryonic development. Interference contrast images of the developing male genital apparatus in the tail plate of *Macrostomum lignano* during regeneration (**A–C**) and postembryonic development (**D–F**), dorsal view. The scheme in (F) is indicating the orientation of the animals in all subpanels. (A) Three days after amputation of the tail plate, the tip of the male copulatory organ called stylet (arrow), and a small vesicula granulorum (arrowheads) are visible within the blastema. (B) Four days after amputation, the stylet is almost completely regenerated, and the first sperm have appeared in the seminal vesicle. (C) Five days after amputation, the stylet has grown to full size and the seminal vesicles are full of sperm. (D) Stylet (arrow) with vesicula granulorum (arrowheads) in the tail plate of an 8-day-old hatchling. (E) Stylet in the tail plate of an 8-day-old hatchling with more pronounced stylet connected to the vesicula granulorum. (F) Eleven-day-old hatchling with full-grown stylet and sperm-filled seminal vesicles. fsv, false seminal vesicle; gv, granular vesicle; st, stylet; vs, seminal vesicle. Scale bar for all subpanels is 20 μm .

produce an additional head in an adult is severely restricted. Otherwise, obtaining double-headed flatworms by lateral incision would be akin to producing double-headed humans by inflicting deep wounds, e.g., at the shoulder region.

Is the largely lost regeneration capacity in humans linked with the loss of such potent neoblast-like cells during embryonic development? It is probably not as straightforward as that. Some fish and amphibians can at least

regenerate tails or limbs, but rely on dedifferentiation rather than totipotent stem cells (Akimenko et al., 2003; Morrison et al., 2006). In the end, not only comparison with other vertebrates but also comparison with well-regen-

erating invertebrate groups would be enlightening for understanding why some animals can regenerate, while others cannot.

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REFERENCES

- Akimenko MA, Marí-Beffa M, Becerra J, Géraudie J. 2003. Old questions, new tools, and some answers to the mystery of fin regeneration. *Dev Dyn* 226:190–201.
- Åkesson B, Rice SA. 1992. Two new *Dorvillea* species (Dorvilleidae, Polychaeta) with obligate asexual reproduction. *Zool Script* 21:351–362.
- Baumiller TK, Gahn FJ. 2004. Testing predator-driven evolution with paleozoic crinoid arm regeneration. *Science* 305:1453–1455.
- Bely AE. 2006. Distribution of segment regeneration ability in the Annelida. *Integrat Comp Biol* 46:508–518.
- Bickell-Page LR, Mackie GO. 1991. Tentacle autotomy in the hydromedusa *Aglantha digitale* (Cnidaria): an ultrastructural and neurophysiological analysis. *Phil Trans Royal Soc Lond B* 331:155–170.
- Bierne J. 1990. *Lineus* as a model for studying developmental processes in animals reconstructed from adult pieces. *Int J Dev Biol* 34:245–253.
- Brøndsted HV. 1969. Planarian regeneration. Oxford: Pergamon Press.
- Bulliere D, Bulliere F. 1985. Regeneration. In: Kerkut GA, Gilbert CI, editors. *Comprehensive insect physiology, biochemistry and pharmacology*. Vol 2. Oxford: Pergamon Press. pp. 371–424.
- Cardona A, Hartenstein V, Romero R. 2005. The embryonic development of the triclad *Schmidtea polychroa*. *Dev Genes Evol* 215:109–131.
- Child CM. 1914. Starvation, rejuvenescence and acclimation in *Planaria dorotocephala*. *Arch Entwicklungsmech Org* 38:418–446.
- Coe WR. 1929. Regeneration in nemerteans. *J Exp Zool* 54:411–460.
- Cooper WE. 2003. Shifted balance of risk and cost after autotomy affects use of cover, escape, activity, and foraging in the keeled earless lizard (*Holbrookia propinqua*). *Behav Ecol Sociobiol* 54:179–187.
- Dalyell JG. 1814. Observations on some interesting phenomena in animal physiology exhibited by several species of planariae. London, UK: Edinburgh House.
- De Vlas J. 1979. Secondary production by tail regeneration in a tidal flat population of lugworms (*Arenicola marina*), cropped by flatfish. *Neth J Sea Res* 13:362–393.
- Dial BE, Fitzpatrick LC. 1984. Predator escape success in tailed versus tailless *Scincella lateralis* (Sauria: Scincidae). *Anim Behav* 32:301–302.
- Egger B, Ladurner P, Nimeth K, et al. 2006. The regeneration capacity of the flatworm *Macrostomum lignano*—on repeated regeneration, rejuvenation, and the minimal size needed for regeneration. *Dev Genes Evol* 216:565–577.
- Egger B, Gschwentner R, Rieger R. 2007. Free-living flatworms under the knife: past and present. *Dev Genes Evol* 217:89–104.
- Fischer A. 1999. Reproductive and developmental phenomena in annelids: a source of exemplary research problems. *Hydrobiologia* 402:1–20.
- Fleming PA, Muller D, Bateman PW. 2007. Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biol Rev* 82:481–510.
- Goss RJ. 1991. The natural history (and mystery) of regeneration. In: Dinsmore CE, editor. *A history of regeneration research: milestones in the evolution of science*. Cambridge: Cambridge University Press, p. 7–23.
- Han M, Yang X, Lee J, et al. 2008. Development and regeneration of the neonatal digit tip in mice. *Dev Biol* 315:125–135.
- Haranghy L, Balázs A. 1964. Ageing and rejuvenation in planarians. *Exp Gerontol* 1:77–91.
- Hendelberg J, Åkesson B. 1988. *Convolutriloba retrogemma* gen. et sp.n., a turbellarian (Acoela, Platyhelminthes) with reversed polarity of reproductive buds. *Fortschr Zool* 36:321–327.
- Hendelberg J, Åkesson B. 1991. Studies of the budding process in *Convolutriloba retrogemma* (Acoela, Platyhelminthes). *Hydrobiologia* 227:11–17.
- Kato C, Mihashi K, Ishida S. 2004. Motility recovery during the process of regeneration in freshwater planarians. *Behav Brain Res* 150:9–14.
- Keller J. 1894. Die ungeschlechtliche Fortpflanzung der Süßwasserturbellarien. *Jena Z Naturwiss* 28:370–407.
- Koopowitz H, Silver D, Rose G. 1976. Primitive nervous systems. Control and recovery of feeding behaviour in the polyclad flatworm, *Notoplana acticola*. *Biol Bull* 150:411–425.
- Ladurner P, Egger B, De Mulder K, et al. 2008. The stem cell system of the basal flatworm *Macrostomum lignano*. In: Bosch TCG, editor. *Stem cells—from hydra to man*. Netherlands: Springer Verlag. pp.75–94.
- Lang A. 1884. Die Polycladen des Golfes von Neapel und der angrenzenden Meeresabschnitte. Eine Monographie. Leipzig: Fauna and Flora Golfes Neapel. pp. 1–668.
- Lus J. 1924. Studies on regeneration and transplantation in turbellaria. I. Some considerations on polarity and heteromorphosis in freshwater planarians. *Bull Soc Nat Mosc Sect Biol, Exp I*.
- Maginnis TL. 2006. Leg regeneration stunts wing growth and hinders flight performance in a stick insect (*Sipyloidea sipyilus*). *Proc R Soc B* 273:1811–1814.
- Martínez DE. 1996. Rejuvenation of the disposable soma: repeated injury extends lifespan in an asexual annelid. *Exp Gerontol* 31:699–704.
- Montgomery JR, Coward SJ. 1974. On the minimal size of a planarian capable of regeneration. *Trans Am Microsc Soc* 93:386–391.
- Morgan TH. 1902. Growth and regeneration in *Planaria lugubris*. *Arch Entwmm* 13:179–212.
- Morrison JI, Lööf S, He P, Simon A. 2006. Salamander limb regeneration involves the activation of a multipotent skeletal muscle satellite cell population. *J Cell Biol* 172:433–440.
- Naya DE, Veloso C, Muñoz JLP, Bozinovic F. 2007. Some vaguely explored (but not trivial) costs of tail autotomy in lizards. *Comp Biochem Physiol A* 146:189–193.
- Pallas PS. 1774. *Spicilegia zoologica quibus novae imprimis et obscurae animalium. Speciosiconibus atque commentariis illustratur*. Berolini: Fasc X.
- Rieger R. 1996. Plathelminthes, Plattwürmer. In: Westheide W, Rieger R, editors. *Spezielle Zoologie I*. Einzeller und Wirbellose Tiere. Stuttgart: Gustav Fischer Verlag. pp. 210–258.
- Reuter M, Kreshchenko N. 2004. Flatworm asexual multiplication implicates stem cells and regeneration. *Can J Zool* 82:334–356.
- Saló E, Bagaña J. 2002. Regeneration in planarians and other worms: new findings, new tools, and new perspectives. *J Exp Zool* 292:528–539.
- Sánchez Alvarado A. 2000. Regeneration in the metazoans: why does it happen? *Bioessays* 22:578–590.
- Schärer L, Knoflach D, Vizoso DB, et al. 2007. Thraustochytrids as novel parasitic protists of marine free-living flatworms: *Thraustochytrium caudivorum* sp. nov. parasitizes *Macrostomum lignano*. *Mar Biol* 152:1095–1104.
- Toledo A, Cruz C, Fragoso G, et al. 1997. In vitro culture of *Taenia crassiceps* larval cells and cyst regeneration after injection into mice. *J Parasitol* 83:189–193.
- Teshirogi W, Watanabe K, editors. 1998. *Morpho-differentiation in planarians*. Tokyo: Kyoritsu Shuppan Company. pp. 223–240.
- van Cleave CD. 1929. An experimental study of fission and reconstitution in *Stenostomum*. *Physiol Zool* 2:18–58.
- von Graff L. 1882. *Monographie der Turbellarien. I. Rhabdocoelida*. Leipzig: Verlag Wilhelm Engelmann.