

Appendage loss and regeneration in arthropods: A comparative view

DIEGO MARUZZO, LUCIO BONATO, CARLO BRENA, GIUSEPPE FUSCO & ALESSANDRO MINELLI

Department of Biology, University of Padova, Padova, Italy

ABSTRACT

Evidence for loss and regeneration of arthropod appendages is reviewed and discussed in terms of comparative developmental biology and arthropod phylogeny. The presence of a preferential breakage point is well documented for some, but not all, lineages within each of the four major groups - chelicerates, myriapods, crustaceans and hexapods. Undisputed evidence of true autotomy, however, is limited to isopods, decapods and some basal pterygotes, and claimed for other groups. Regeneration of lost appendages is widespread within arthropods, even if not present or documented in some groups. During regeneration, growth and differentiation of epidermis, nerves, muscles and tracheae are to some extent mutually independent, thus sometimes failing to reproduce their usual developmental interactions, with obvious consequences on the reconstruction of the lost part of the appendage. In the regeneration of appendages composed of 'true segments', all the segments the animal is able to regenerate are already present (with extremely rare exceptions) following the first post-operative molt, whereas the regeneration of flagellar structures is often accomplished in steps, e.g., the first regenerate may show a reduced number of flagellomeres. Lack of autotomy is likely to be the plesiomorphic condition in arthropods, a condition maintained in the Myriochelata (myriapods plus chelicerates). Autotomy evolved within the Pancrustacea, perhaps close to the origin of a Malacostraca-Hexapoda clade, and was subsequently lost by some lineages, e.g., the Hemipteroidea and the endopterygote insects. A diaphragm reducing the risk of hemorrhage at the preferred breakage point of the appendage is generally associated with autotomizing appendages, but this anatomical specialization has been lost in some groups, including one (the Dictyoptera) where autotomy is still present.

This article is dedicated to Fred Schram in deep appreciation of his lasting contribution to arthropod phylogeny and comparative morphology, with the warmest wishes of the authors.

1 INTRODUCTION

Studies on regeneration were fashionable one hundred years ago, when developmental biology was turning from a merely descriptive into an experimental science, but was technically and conceptually limited to mechanical approaches. This was the time of the *Ent-*

wicklungsmechanik (mechanics of development), with Needham's chemical embryology, not to mention molecular developmental genetics, still decades ahead. Today, new powerful means of investigation are progressively resolving developmental mechanisms in terms of gene expression patterns, transcription factors, and molecular dialogues between cells. As for arthropods, modern studies on regeneration have mostly focused on those species of decapod crustaceans in which leg autotomy, followed by regeneration, is a frequent, natural occurrence.

Most of the bulky descriptive literature produced in the past century on animal regeneration is now ignored. To be sure, many old studies on regeneration lacked clear experimental design and what was reported in print was often little more than a scientifically shallow narrative. There was, however, an important approach in that literature, one that later sank into oblivion and has come back into the focus of front line research only recently, with the advent of evolutionary developmental biology: the comparative approach. Przi Bram's (1909) monograph gives us an idea of the diversity of organisms brought by biologists into the lab to perform experiments on regeneration. It also gives an idea of the propensity of many researchers of that generation to collect and comparatively analyze both experimental evidence and accurate descriptions of museum specimens. This latter aspect, however, which was eventually so productive for other fields of biology - as in Bateson's (1894) masterpiece, *Materials for the study of variation* - is one of the weaknesses of the old literature on regeneration. Reports were very often based on observation of field-caught specimens rather than on experimentation. Incomplete regeneration was the default hypothesis to explain the origin of defective appendages, e.g., those with segments reduced in number or size. Alternative explanations - defective embryonic or post-embryonic development without any traumatic removal of the original appendage, or of part of it - were seldom advanced. With hindsight, and with the benefit of better knowledge of arthropod development, we are sometimes able to evaluate the likelihood of that putative evidence of regeneration, but the reliability of many records remains nevertheless uncertain. This is mainly due to the fact that our current awareness of developmental processes is primarily limited to a few model species, from which we cannot safely generalize for all arthropods, as this review will show.

In conjunction with the experimental work on the regeneration of arthropod appendages recently started in our lab, we felt the need to systematically explore the literature on this subject, in order to summarize a scattered, unequal but nevertheless precious trove of comparative information that has not been re-evaluated in the context of evolutionary developmental biology.

In our summary of data about regeneration in different groups, we primarily focus on the walking legs; data on the remaining appendages is given whenever available. Our choice is a consequence of the prevailing focus of regeneration research in most arthropod groups. It should not be construed as depending on a concept of the walking leg as the default arthropod appendage, from which all other kinds are derived, a concept one of us has recently refuted (Minelli 2003a).

1.1 *Nomenclature*

In the following review, we will often mention the presence, or absence, of a preferred

breakage point (also called 'autotomy plane' in the literature), using the acronym PBP.

The term appendotomy will be used for any loss of a more or less extended distal section of an arthropod appendage at the level of a PBP. Many authors used the term autotomy as a synonym of appendotomy, but a more restricted use of the former is recommended (Bliss 1960; Roth & Roth 1984). Following Bliss (1960), we will speak of autotomy when appendotomy is produced "by means of a reflex that is usually unisegmental", autospasy "when the appendage is pulled by an outside agent against resistance provided by the animal's weight or its efforts to escape" and autotilly if it occurs "with the assistance of mouthparts, claws, or walking legs of the animal itself."

1.2 *The scope of the present review*

We have mainly limited our attention to the following points: presence (and, if any, relative position along the appendage) of a preferred breakage point (PBP); occurrence of true autotomy (see above, par. 1.1); natural occurrence of regeneration following appendotomy; occurrence of regeneration following experimental or accidental amputation of a distal part of the appendage; dependence of regeneration on the proximo-distal level of the breakage and, in particular, relative to the PBP; timing of regeneration process, in terms of number of molts required to get a regenerate, or to complete its growth and differentiation. As far as information is available, we have also paid attention to the sequence with which the segments of the regenerating appendage differentiate and to the possibly different process of regenerating 'true segments' in comparison with regenerating flagellar structures.

Several important features of arthropod appendage regeneration are deliberately omitted from the present review. In particular, we will not report on the detailed histological information available for some regenerating appendages. Other aspects we will not cover are hormonal or nervous control, regeneration following experimental grafting, and heteromorphic regeneration (for example, an antenna replacing the eye-stalk of a decapod crustacean, or a leg-like regenerate replacing the antenna of a stick insect).

As to the adaptive value of regeneration, we shall only mention the very high frequency of specimens with regenerated appendages sometimes found in natural populations (up to 40% in some brachyuran crustaceans). Besides its obvious importance as a means to restore accidentally damaged appendages resulting from intraspecific fighting or from a foe's offence, regeneration is sometimes a complement of an autotomy mechanism that provides an excellent way to escape from predators or to get rid of a riotous exuvium. On the other hand, it would also be interesting to analyze how much the different regenerative behavior of different appendages within one and the same animal (e.g., decapod chelipeds versus walking legs, or orthopteran forelegs versus hindlegs) can be explained by 'phylogenetic inertia', and how much instead by current functional constraints. We will only briefly discuss the 'assimilation' of autotomy followed by regeneration into the regular ontogenetic schedule of some male fiddler crabs, to the extent that these events are required to obtain functional heterochely. It would be worthwhile, but again outside the scope of the present review, to also discuss considerations of resource allocation during regeneration, and how they affect growth rate and frequency of later molts. Finally, we will leave out of the picture the developmental events that depend on growth and differentiation of imaginal discs; therefore, our brief consideration of holometabolous insects will be limited to the regenera-

tion of their larval appendages.

Despite our choice to focus strictly on selected comparative aspects, the evidence presented in the following section will reveal many suggestive patterns, briefly discussed in the final section both from a phylogenetic and a comparative developmental perspective.

2 A SURVEY BY MAJOR TAXA

2.1 *Pycnogonida and Chelicerata*

2.1.1 *Pycnogonida*

Appendotomy has been observed in the basal region of the legs of *Phoxichilus*. The gut branch extending into the broken leg is left undamaged and is eventually cut off by the new epidermis growing in from the leg stump. In *Nymphon*, PBP is between the first and the second segment of the leg. In both species, appendotomy is followed by regeneration (Dohrn 1881; Gaubert 1892).

In some pycnogonids, e.g. *Colossendeis*, the chelicerae are deciduous and fall off during the last pre-adult instar (Kaestner 1968; Bain 2003). This behavior could be described as appendotomy without regeneration.

2.1.2 *Xiphosura*

The appendages of *Limulus* do not show any trace of PBP (Wood & Wood 1932); however, telson and limbs can regenerate, at least during the larval stages (Clare et al. 1990 and references therein).

2.1.3 *Scorpiones*

Based on the experimental work of Wood (1926) on *Centrurus*, scorpion appendages do not show a PBP.

Scorpions seem to be able to regenerate only the pretarsus, which can arise from whatever level at which the leg has been cut. At first, the regenerate is often smaller than the original pretarsus, but can attain full size following more molts. The segment proximal to it can develop traits characteristic of the missing segments, such as the number of sensory setae and the presence of the spine-like setae usually restricted to basitarsus and tarsus (Rosin 1964). The only documented case of regeneration of anything more than just the pretarsus is Vachon's (1957) report on a specimen that, on the second molt after the amputation made in the first post-embryonic ('larval') stage, dissociated its regenerated pretarsus into tarsus and pretarsus. By contrast, Rosin (1964) did not report any further increase of segments in the regenerating limb during subsequent stages (amputation made at nymphal stages).

Following Rosin (1964), scorpions are also able to regenerate the tip of the sting and the distal part of the chelicerae. Both Vachon (1957), who worked with 'larvae' of *Euscorpius carpathicus*, and Rosin (1964), who worked with nymphs of different species, complained high mortality after removal of appendages.

2.1.4 *Opiliones*

A PBP has been found at the trochanter-femur articulation of the species of harvestmen thus far investigated (Wood 1926; Roth & Roth 1984).

In *Leiobunum nigropalpi*, appendotomy does not damage any muscles and the resulting wound is very small and easily clotted. It takes just a slight tension to separate the leg at this point, but there is no evidence of any specialized structure (Wood 1926) to produce autotomy. A second PBP has been reported for *Sclerobunus* near the base of the femur, but apparently, it is seldom used (Roth & Roth 1984).

Even though appendotomy is frequent, it is traditionally thought that harvestmen are unable to regenerate limb segments, despite some old, indirect evidence, as summarized in Przibram (1909).

2.1.5 Acari

Most mites do not have a PBP (Rockett & Woodring 1972), but in *Opilioacarus* (Notostigmata) the legs are easily detached between coxa and trochanter (Vitzthum 1943).

The regenerative power of mites is very poor and only the ticks (Ixodida) (Rockett & Woodring 1972; Belozero 2001) and *Opilioacarus* (Coineau & Legendre 1975) are known to regenerate a missing limb completely. Among the mites, in some species, the site of amputation becomes the definitive end of the appendage; in others, there can be an unpredictable degree of reduction of the segmentation in the remaining segments, which sometimes become smaller and distorted. In most of the operated specimens of *Schelorbates nudus*, the distorted distal segments ended in a long terminal seta and in *Fuscuropoda agitans* there was regeneration of one (very rarely two) small distorted segment(s) with modified setal patterns (Rockett & Woodring 1972).

Again, in mites other than ticks, mortality following leg amputation is fairly high, possibly dependent on slow coagulation of the hemolymph: in *Tetranychus neocaledonicus* and *Pimeliaphilus podapolypophagus*, for which the highest mortality has been reported (more than 90% and 100%, respectively), there seems to be almost no coagulation (Rockett & Woodring 1972).

Ticks are the only mites that can regenerate a full appendage (both legs and mouth parts; Belozero 2001), but there are differences between hard ticks (Ixodidae) and soft ticks (Argasidae) (Rockett & Woodring 1972; Belozero 2001). Hard ticks have lower mortality and faster regeneration since they are able to reproduce a complete limb after just one molt (actually, some differences remain in Haller's organ, in the number and topography of sensilla and some other details; Belozero 2001). In soft ticks, on the other hand, a limb with the complete number of segments, but with reduced size and chetotaxy, emerges at the first molt after amputation; it will require two to four molts to eventually obtain full size (Belozero 2001).

Rockett & Woodring (1972) stated that amputations made in hard ticks in the quiescent stage always resulted in death, but in later experiments (reviewed in Belozero 2001) on both engorged larvae and nymphs, mortality was low and regeneration strongly dependent on the time of amputation within the instar. In soft ticks, amputation during apolysis results in the absence of regeneration (Belozero 2001).

Neither the site of amputation, the number of amputated legs, nor the time of amputation within a given instar, excluding amputations on quiescent stages or during apolysis, seem to influence the regeneration process (Rockett & Woodring 1972; Belozero 2001).

Interestingly, the only mites that exhibit mitoses during post-larval life are the ticks, but few species of mites have been studied in this respect and no data are available for *Opilioacarus*. This suggests a strong association between regeneration and the presence of post-

larval mitoses (Rockett & Woodring 1972).

2.1.6 *Amblypygi*

There is a PBP at the patella-tibia articulation both in the forelegs (the whips, with flagellar tibia and tarsus) and in the walking legs. The anatomy of this joint has been studied in detail by Wood (1926) in *Tarantula*. This joint lacks strength of articulation and interlacing chitin fibers as usually present in the other joints and only one muscle is disturbed, but not injured, during breakage. When the leg is torn apart, this muscle, which arises in the patella, loses its distal attachment, but remains intact, and retracts within the patella, including the distal tip.

Autotomy is not known, whereas leg autospasy has been described by Weygoldt (1984).

Regeneration is possible only at the PBP: there is no regeneration following breakage at points proximal to it, whereas amputations distal to the PBP trigger appendotomy, followed by regeneration (Weygoldt 1984; Igelmund 1987). The walking legs take just one molt to gain full size and the complete number of segments. At the first molt, the regenerating whips are smaller, but with a higher number of segments than their undamaged counterparts. The following molts allow them to obtain full size, but they maintain their extra number of segments (Weygoldt 1984; Igelmund 1987). In *Heterophrynus elaphus*, the number of segments in the regenerated tibia increases by approximately 60%, in the tarsus about 30%. This number might be age-dependent, since regenerated whips from younger animals seem to have fewer segments than those from adults (Igelmund 1987).

2.1.7 *Araneae*

Depending on the species, spiders can have a PBP in the coxa-trochanter articulation, in the patella-tibia articulation or at mid-length on the patella, or no PBP at all (for a review and a list of species, see Roth & Roth 1984). Some species seem to have more than one PBP (Roth & Roth 1984), which would confirm Wood's (1926) concept of a PBP in arachnids that is a simple point of structural weakness.

The structure of the PBP has been studied in detail only for species that have it at the coxa-trochanter articulation; only one muscle is damaged after parting at this point and the opening left is small (Wood 1926).

A PBP also exists in the palps, at least in some species. In *Tidarren*, for example, the males usually self-remove (autotilly) one of their palps at the coxa-trochanter joint soon after their last molt (Roth & Roth 1984; Knoflach & van Harten 2000).

Whether spiders exhibit real autotomy has long been questioned and seems unlikely (Wood 1926; Roth & Roth 1984). Spiders regenerate chelicerae, palps, labium, legs and even the spinnerets (Bonnet 1930; Mikulska et al. 1975). A comprehensive list of early studies about regeneration of legs and palps in different spiders is found in Przibram (1909).

In *Dolomedes* and *Tegenaria*, the regenerate is, at first, shorter and has less than the full complement of sensory hairs; nevertheless, it already possesses all segments (Bonnet 1930; Mikulska et al. 1975).

Vachon (1956, 1967) amputated the legs of *Coelotes terrestris* at a very early stage, the 'larva', when appendages are still incompletely segmented. The first regenerate exhibited reduced segmentation and increased its segment number during subsequent molts, as it would have done in undisturbed development.

The relationship between PBP and regeneration is varied. Some spiders, but not all, regenerate from the PBP as well as from any point distal to it (Bonnet 1930; Mikulska et al. 1975). No data are available about regeneration from levels proximal to the PBP. *Latrodectus variolus* has a PBP at the coxa-trochanter articulation, but regenerates only following amputation in the middle of the femur or distal to that point. However, despite its good regeneration power, it shows appendectomy without regeneration (Randall 1981). Lack of regeneration from the PBP has also been recorded for several other spiders (Vollrath 1990).

There may be differences between regenerated and undamaged appendages in the number of teeth on the leg claws (Bonnet 1930; Mikulska et al. 1975), of sensory setae (Bonnet 1930; Mikulska et al. 1975) and lyriform organs, but not always (Bonnet 1930; Vollrath 1995).

2.2 Myriapoda

2.2.1 *Scutigera* morpho

The long legs of *Scutigera* have a PBP between coxa and trochanter (Verhoeff 1902-1925); no muscle stretches through this articulation, just one nerve does and a diaphragm prevents excessive loss of blood (Herbst 1891).

In *Scutigera*, appendectomy occurs by autotilly or autospasy, whereas autotomy is unlikely (Cameron 1926). Regeneration always starts from the PBP since any cut distal to it results in appendectomy (Cameron 1926).

The regenerating leg is already complete as soon as it appears, after the first post-operative molt or a molt later, depending on the timing of the amputation within the intermolt (Verhoeff 1902-1925; Cameron 1926).

2.2.2 *Lithobiomorpha*

A PBP along the leg is found between coxa and trochanter (Verhoeff 1902-1925).

In *Lithobius*, Verhoeff (1902-1925) described the leg regenerating from the PBP as consisting at first of prefemur, femur, tibia and a tarsus of one article and completely lacking setae, epidermal glands, muscles (these are just 'sketched') and tendons. With another molt, the appendage becomes longer (about half the length of an undamaged leg) and possesses a trochanter, a second tarsal article, a claw and its tendon. The musculature is also developing, although it is still gracile. Many sensory setae and epidermal glands have appeared too, but most of the spines are still missing. A further molt leads to a complete appendage that is slightly smaller than an undamaged one. Regeneration of legs is also possible from any level distal to the PBP. The mechanism does not seem to be the same in all species and/or stadia. In *Bothropolys asperatus*, for instance, the regenerating legs have, at first, an incomplete number of segments if the damage occurred in a larva, but the full number of segments if it was suffered by a post-larval specimen (Murakami 1958).

The antennae can regenerate too; their segment number usually increases with subsequent molts. The number of segments shown after the first post-operative molt not only depends on the point of amputation, but also on the instar and the intermolt stage at the time of the operation (Verhoeff 1902-1925; Scheffel 1987, 1989; Weise 1991).

2.2.3 *Scolopendromorpha*

A PBP is present between coxa and trochanter, at least in the last pair of legs.

Autosphy in the last pair of legs was reported in *Rhysida* (Cloudsley-Thompson 1961), appendectomy in *Cryptops* and *Alipes* (Lawrence 1953).

Regeneration of the legs has never been documented through experiment, but indirect evidence of regeneration of the last pair of legs (reduced size and incomplete armature of spines) has long been noted (Newport 1844). No comparable data are available for the remaining legs.

As for the antennae, the species with a fixed number of antennomeres can only increase the length, but not the number of the segments left after amputation. True regeneration, with an increasing number of segments, seems only possible in those species that usually add a few antennomeres during post-embryonic development (Lewis 2000). In *Scolopendra*, Lewis (1968) observed antennae composed of a few proximal antennomeres of expected size followed by a variable number of very short ones and interpreted those distal articles as regenerated. In this group, the regeneration of antennomeres is far from accurate, sometimes leading to atypically high numbers (Lewis 2000).

2.2.4 *Geophilomorpha*

No evidence is available for the presence of a PBP and the occurrence of regeneration in geophilomorph legs and antennae (Lewis 2000; Minelli et al. 2000). A vague reference to appendectomy of the last pair of legs is found in Lawrence (1953).

2.2.5 *Diplopoda*

Appendectomy apparently does not exist in this group (Lawrence 1953).

In an early study, Newport (1844) reported on regeneration in two chilognathan genera he referred to as “*Julus*” and “*Spirostreptus*”. Legs regenerated with all the articles at the first post-operative molt, but with reduced size; it is not known how much their size increased with subsequent molts. The antennae also regenerated, but, after the first post-operative molt, both number and size of their articles could be defective depending on where the amputation occurred. It is not known if the number of segments increased with subsequent molts.

2.3 *Crustacea*

2.3.1 *Branchiopoda*

In this group there is no evidence of any PBP.

Regeneration of appendages was observed in anostracans (*Branchipus*: Przibram 1909) and notostracans (*Lepidurus*), particularly in endites of the thoracic legs (Rogers 2001). By contrast, the antennae of cladocerans (*Daphnia*, *Simocephalus*, *Ceriodaphnia*) never regenerated their articulated axis if experimentally cut, but only reproduced their complex system of ‘muscular setae’ (Agar 1930). It is worthwhile to notice that the regenerated setae are not as morphologically and functionally diversified as the original setae. Regenerated setae, whose number and size increase at each postoperative molt, are variable among specimens in both number and features, and are not affected by factors such as age, food availability and number of repeated operations (Agar 1930).

Regeneration of furcal rami was also reported in notostracans (*Apus = Triops*) (Rabes 1907). The number of flagellar units increases in successive molts, and the size of the regenerating filament converges toward that of the undamaged one.

2.3.2 Ostracoda, Copepoda, Cirripedia, Branchiura

Based on the scarce information available, including Wood & Wood's (1932) experimental work on *Lepas*, no PBP has been documented in these crustacean groups.

Przibram (1909) summarized limited early evidence of regeneration of antennae and furcal rami in cyclopid and diaptomid copepods. As for cirripedes, regeneration was reported in *Balanus* for the cirri (Darwin 1854) and the penis (Klepal & Barnes 1974). No evidence of appendage regeneration is available for branchiurans but for the puzzling record of a specimen of *Argulus* that produced two extra pairs of functional, thoracic-like legs following the ablation of the abdomen (Kocian 1930).

2.3.3 Phyllocarida and Stomatopoda

No PBP has been documented in these groups but for Wood & Wood's (1932) weak experimental evidence of a possible PBP at the joint between ischium and merus in *Squilla*.

Regeneration of antennae and furcal rami was reported in *Nebalia* (Przibram 1909). It is worth noting that the heteromorphic regeneration of the eye-stalk as a functional antennule was reported in *Squilla* (see Paulian 1938).

2.3.4 Isopoda

No experimental evidence of a PBP was found by Wood & Wood (1932) in the isopods *Oniscus*, *Cylisticus*, *Lygia* and *Sphaeroma*, but the presence of a PBP is documented in the legs of *Asellus* (Needham 1947) and in the proximal part of the basis in the legs of *Porcellio* (Noulin 1962, 1984). This PBP is not crossed by muscles and possesses a diaphragm (Noulin 1962; Needham 1965). A PBP, apparently without diaphragm, was also observed in the antennae of *Asellus* (Wege 1911), between the third and the fourth article.

Autotomy of the legs has been documented in some isopods (*Asellus*: Needham 1947; *Porcellio*: Noulin 1962), but could not be observed in other taxa (Wood & Wood 1932).

Regeneration has been documented in different kinds of isopod appendages, but in different species (for brief summaries see Przibram 1909; Vernet & Charmantier-Daures 1994).

The mechanism of leg regeneration was studied by Needham (reviewed in 1965) in *Asellus*: after the formation of a scab, a folded, regenerating limb is produced inside a cuticular sac and the regenerate appears following the next molt.

In the regenerating antenna of *Asellus*, the number of segments increases through successive molts, converging with the number in the undamaged appendage; the peduncle and the most distal part of the antennal flagellum are formed first, followed by more flagellomeres in between (Wege 1911).

2.3.5 Amphipoda

A PBP was not found by Wood & Wood (1932) in the legs of *Gammarus*, *Caprella* and *Orchestia*, but a point of less resistance seems, nevertheless, to exist both in the caprellids (Calman 1909) and in *Orchestia* (between basis and ischium; Charniaux-Cotton 1957).

Regeneration was documented for legs (different species, see Przibram 1909), antennae

(*Gammarus*: Dixey 1938; Paulian 1938), and the second pair of gnathopods (*Orchestia*: Charniaux-Cotton 1957). In this latter case, gnathopods are sexually dimorphic and the regeneration of the derived male appendage proceeds through an intermediate stage similar to the condition in juveniles and in adult females (Charniaux-Cotton 1957). Timing of regeneration and completeness of the regenerate depend on the intermolt stage at the time of amputation (reviews in Bliss 1960; Vernet & Charmantier-Daures 1994).

2.3.6 *Decapoda: Brachyura*

The existence of a PBP along the leg was well documented in almost all of the investigated species of brachyurans (reviews in Wood & Wood 1932; Bliss 1960). The PBP is most often localized at the joint between basis and ischium, which is usually a non-functional articulation (Wood & Wood 1932; Bliss 1960). This PBP, however, is functionally weak or even not detectable at all in some fossorial species, such as *Ranina ranina*, probably as a derived condition (Wood & Wood 1932; Juanes & Smith 1995).

The anatomical structure of the PBP was accurately studied in some representative brachyurans (Wood & Wood 1932; Bliss 1960; Adiyodi 1972). Microcanals spanning the whole depth of the cuticle are more abundant here than in other regions of the appendage. No muscle develops through the PBP; instead, a specialized "autotomizer" muscle is inserted just proximal to it. The only nerve developing through the PBP is locally tapered and, thus, weakly resistant and the blood vessels crossing the PBP have a valve preventing bleeding.

Autotomy of the legs was well documented in most investigated brachyurans and the mechanism involved was studied in detail in some species (Wood & Wood 1932; review in Bliss 1960). Contraction of the autotomizer muscle pulls the anterior-basal part of the basis into the coxa; the mechanical resistance of the distal margin of the coxa against the anterior surface of the basi-ischium results in breaking of the integument between basis and ischium. The nerve and the blood vessels are cut. Autotomy is infrequent soon after ecdysis, when the exoskeleton is more flexible, possibly depending on the lack of mechanically suitable conditions (Wood & Wood 1932).

Full regeneration of legs from the PBP was documented in most brachyurans, both in nature and under experimental conditions (Bliss 1960; Vernet & Charmantier-Daures 1994). The papilla emerging after the detachment of the scar develops into an external cuticular sac; the limb bud grows and differentiates inside this sac, as a double-folded limb, during the intermolt period. An early phase of fast growth and articular differentiation of the regenerating limb, involving mitotic proliferation, is usually followed by a temporary developmental stasis, which is variable in duration in relation to the intermolt cycle. After a further phase of growth, mainly involving protein and water accumulation, at the first molt the regenerating limb emerges out of the cuticular sac. According to Adiyodi (1972), in *Paratelphusa*, an intermediate segment (the merus) is the first to develop during the differentiation of the regenerating leg, followed by the remaining segments.

An efficient and quite rapid regenerative process is known for the five pairs of pereopods when these appendages are detached at the level of their PBP. Efficiency and rapidity, however, are often different for the different pairs of legs of the same specimen and were found to be influenced by both internal factors such as the developmental stage and the physiological status of the specimen (Paulian 1938; Spivak & Politis 1989; Juanes & Smith 1995), and external factors such as environmental temperature, water salinity and concen-

tration of some chemicals (Bliss 1960); also population density was found to affect the regenerative process (Juanes & Smith 1995).

Apart from the regeneration associated with the PBP, regenerative processes are also known from points distal to the PBP or even proximal to it. Lost dactyli of the chelipeds, for instance, can regenerate through the formation of a swollen hard tip, within which a new dactylus grows until its emergence at the first molt (Bliss 1960). In all these cases, however, growth and differentiation are slower and less efficient than regeneration from the PBP and the regenerate is not always complete.

Brachyurans are able to regenerate more than one leg at the same time. Autotomy of more than one leg occurs frequently in free-living specimens, and in these cases, all appendages undergo regeneration. Sometimes, when more than one leg is affected, the frequency of molts is increased. The growth of regenerating appendage(s) can limit the growth of intact limbs, particularly when several appendages are regenerating at the same time (Hopkins 1985). When different appendages are detached at different times, hormone-controlled regulative processes have been observed to synchronize the growth schedules of the regenerating appendages (Skinner 1985; Mykles 2001).

Regenerated legs show the same properties as the original legs in terms of ability to (re)autotomize and regenerative potential. The PBP, in particular, is reproduced very early during the regenerative process and appendotomy can be demonstrated well before the regeneration of the appendage is completed (Hopkins 1993). Thus, a leg may regenerate more than once (e.g., McConaugha 1991).

The regenerate acquires full size usually within two or three molts after detachment, but sometimes requires only one molt (e.g., Ameer Hamsa 1982).

In heterochelous brachyurans, the asymmetric pair of chelipeds is composed of two morphologically and functionally different limbs. The regeneration of one of these appendages, e.g., left or right cheliped, can affect the asymmetric condition. In some species (e.g., some *Uca*), regeneration produces a cheliped that is subequal to the original limb and the asymmetric pattern is maintained (e.g., Morgan 1920, 1923; Yamaguchi 2001). However, in other species of *Uca* the regenerate may be either subequal to the original cheliped or of the opposite type, depending on the developmental stage when regeneration occurs and on the reciprocal regulation between the two chelipeds. In some species of *Uca*, indeed, appendotomy and subsequent regeneration of a cheliped of the first pair is a regular event during male development and seems to be required to release differentiation of two asymmetric chelipeds, something of vital importance to the adult male crab (Hartnoll 1988; Yamaguchi 2001).

2.3.7 *Decapoda other than Brachyura*

A PBP at the joint between basis and ischium of the legs was documented in some, but not all, of the investigated species of non-brachyuran decapods (reviews in Wood & Wood 1932; Bliss 1960). This PBP is functionally weak or even not detectable at all in some species with morphological and behavioral adaptations to a fossorial life, such as the anomurans *Hippa* and *Emerita talpoidea*, as a probably derived condition (Wood & Wood 1932; Weis 1982; Juanes & Smith 1995). Instead, a different PBP, one at the joint between coxa and basis, was documented in *Hippa* (Wood & Wood 1932) and possibly also in the palinuran *Willemoesia* (Calman 1909).

A PBP was also documented in the antennae, between third and fourth article, in some

species at least (e.g., *Palinurus*; Wood & Wood 1932). Other kinds of appendages such as the uropods, however, seem to lack a PBP (e.g., Toyota et al. 2003).

Autotomy of the legs was well documented in most investigated anomurans, as well as in some other non-brachyuran decapods (e.g., Wood & Wood 1932). However, the autotomic properties are often different among different pairs of legs: in some Astacidea (e.g., *Homarus*, *Cambarus*) and Thalassinidea (*Gebia* = *Upogebia*), autotomy was documented for the first cheliped pair only, while only autospasy was found in the walking legs. In some pagurids, autotomy was documented for all the three anterior pairs of pereopods, but only autospasy for the two posterior (reduced) pairs; in other decapods (e.g., *Palinurus* and *Galathea*), similar autotomic properties were reported for all five pairs of pereopods (Wood & Wood 1932; review in Bliss 1960).

Full regeneration of legs from the PBP was well documented in many non-brachyuran decapods (Bliss 1960; Vernet & Charmantier-Daures 1994), as was the regeneration of the antennae (e.g., in *Procambarus*: Mellon & Tewari 2000). A comprehensive list of early studies on the regeneration of different appendages in a number of decapod species was presented by Przibram (1909).

In anomurans the process of regeneration is very similar to that observed in brachyurans (see under 2.3.6), the limb bud growing and differentiating inside an external cuticular sac and emerging only later. In other decapods, conversely, an external limb bud emerges early after the detachment of the scab formed at the PBP; growth and structural differentiation of the limb proceed gradually during the intermolt period; in particular, the chela develops from a longitudinal furrow at the tip and the articular joints develop from annular, transversal furrows along the limb. As to the temporal sequence during which the different joints appear, there is disagreement among reports that deal with different species (e.g., Nouvelvan Rysselberge 1937; Bliss 1960; Govind & Read 1994; Read & Govind 1997a, b). It is not clear how much this reflects actual interspecific differences rather than the different quality of the studies.

In the king crab *Paralithodes camtschatica*, the regenerate requires four to seven molts, and up to seven years, to recover full size (Niwa and Kurata 1964; Edwards 1972).

Regenerative processes have been documented also from points different from the PBP as well as in appendages lacking any PBP (e.g., Bliss 1960). Experimental work on the uropods of *Marsupenaeus* documented high variability in the shape of the outgrowths produced after removing these appendages (Toyota et al. 2003). Ablation of eye stalks, which is commonly practiced in industrially reared decapods, usually does not induce any regenerative process. However, sometimes it results in heteromorphic regenerates and occasionally a full-size and structurally complete eye stalk can be reproduced (*Penaeus*: Desai & Achuthankutty 2000).

The effect of appendectomy and regeneration of one of the two heterochelous chelipeds on the heterochely of the same pair of appendages has been investigated in some non-brachyurans decapods (Govind & Read 1994; Read & Govind 1997a, b; Mariappan et al. 2000). Research on *Alpheus* documented high plasticity in the regenerative program and the regulative role of the underlying asymmetric nervous ganglia. In particular, the original asymmetric pattern of the first pair of chelipeds may be completely reversed or even changed into a symmetric pattern, producing a pair of subequal chelipeds (Govind & Read 1994; Read & Govind 1997a, b).

2.4 Hexapoda

2.4.1 Collembola

No evidence is available about a possible PBP.

Little is known about regeneration. The antennae of *Orchesella*, *Tomocerus*, *Folsomia* and *Heteromurus* can regenerate, but never obtain full size and usually regenerate just one segment, sometimes two. The regenerated segment(s) can be longer than the original one(s) (Ernsting & Fokkema 1983).

2.4.2 Diplura

The cerci of *Campodea* seem to show a PBP and, thus, some kind of appendotomy (Condé 1955).

Regeneration occurs in the antennae (Condé 1955): in the regenerate, the distal-most segment is much longer than in the original appendage (Condé 1955). Regeneration is also possible in legs (Lawrence 1953).

2.4.3 Archaeognatha and Zygentoma

The maxillary palps and legs of machilids usually break at a PBP (Wygodzinsky 1941).

Regeneration of the antennal segments, of the three posterior appendages and at least tibia and tarsus of the legs has been recorded in *Machilis* (Przibram & Werber 1907) and in *Thermobia* and *Lepisma* (Sweetman 1934). The pattern of sensilla in the regenerated palp of *Thermobia* shows irregularities that are not corrected during subsequent molts (Larink 1983).

2.4.4 Ephemeroptera

In the leg of mayflies, there is a PBP between trochanter and femur (Nilsson 1986).

Regeneration occurs, but regenerated legs are small and malformed. If still undersized at the last nymphal instar, the legs fail to grow to full size in the adult (Nilsson 1986). Mayfly nymphs can also regenerate antennae, posterior appendages and lateral gills (Przibram 1909).

2.4.5 Odonata

In zygopterans, the leg has a PBP between trochanter and femur. No muscle crosses this articulation and there is no evidence that it is functionally jointed. A fibrous diaphragm, with a small central gap, separates trochanter and femur. The presence of a large muscle in the trochanter suggests the existence of autotomy (Child & Young 1903).

Zygopterans have high regenerative capabilities. Regeneration (Child & Young 1903) can occur after cuts at any level along the leg. Cuts at different levels along the three-segmented tarsus produce different results. A cut at the base of the most distal tarsomere results in an incompletely regenerated tarsus. Amputation at the base of the second tarsomere results in disintegration of the remaining proximal tarsomere, and the subsequent regeneration from the tibio-tarsal articulation produces a complete tarsus within five or six molts. After the first molt, the tarsus is composed of only one segment and the claws; after the second molt, it has two segments, and after three to four additional molts its full complement of three segments. Amputations in the distal part of the tibia can result in regeneration from the cutting plane, while more proximal cuts, as well as amputations along the

femur, produce appendotomy. Regeneration from the PBP is rapid, but incomplete regarding number of segments and function. After the first molt, the leg has an unsegmented tarsus with unarticulated claws of reduced size and unusual shape. Following the next molt, the tarsus almost always has two segments, but additional molts fail to produce a three-segmented tarsus with functional claws. Regeneration from levels more proximal than the PBP is also possible and follows the same path just described.

Child & Young (1903) observed that, during regeneration, the development of joints is closely correlated with the development of muscle insertions. Their interpretation is that muscles can apparently not keep pace with the growth of the exoskeleton during regeneration, and thus, influence the segmentation process. By contrast, during undisturbed development, the tegumentary structures grow more slowly and muscles can adequately attach to them.

Leg regeneration has been also described in anisopterans (see Przibram 1909). Zygopterans can regenerate the tracheal gills (Child & Young 1903) and in the anisopterans *Anax imperator* and *Aeshna cyanea*, Degrange & Seassau (1974) reported the regeneration of the mask.

2.4.6 *Blattodea*

In the leg of cockroaches, a PBP exists between trochanter and femur, but there is no diaphragm and two muscles cross this articulation (Bordage 1905).

Autotomy is documented in this group (e.g., Penzlin 1963).

Cockroaches have well-developed regenerative capabilities. Regeneration can occur after cuts at any level along the leg. After the first post-operative molt, the regenerating leg is always composed of the final number of segments. However, in *Blaberus craniifer*, amputations along the coxa are followed by a 'two-phase' regeneration, during which an unsegmented bud appears at first. It takes one more molt to produce a segmented leg. In *Blaberus craniifer* and in *Blattella germanica*, experimental amputations distal to the mid-length of the third tarsomere produce a regenerate with the full number of five tarsomeres, while more proximal amputations produce a regenerate with four tarsomeres only (Bullière & Bullière 1985; Tanaka et al. 1992). By contrast, in *Periplaneta americana* and *Panchlora maderae* (= *Rhyparobia maderae*), every amputation made along the tarsus results in the loss of the remaining tarsal segments (apparently, a kind of appendotomy), and the subsequent regeneration begins at the tibia-tarsus articulation. In these species, regeneration invariably produces a four-segmented tarsus (Bordage 1905; Penzlin 1963). Amputation along the femur induces appendotomy in *Blaberus craniifer* (Bullière & Bullière 1985).

The length of the regenerate is correlated with the time of amputation within the inter-molt period (Penzlin 1963). In *Periplaneta americana*, Kaars et al. (1984) observed that in the undamaged femur, nerve and trachea are closely associated and branch together at regular intervals, while in regenerate femurs, nerve and trachea are not closely associated and have a different branching pattern. Their interpretation is that tissue-level interactions during regeneration differ from those during embryogenesis. In *Periplaneta*, wound healing following appendotomy involves cell movement and cell division only in the distal half of the trochanter, while the formation of the blastema involves cell movement and cell division in the temporarily and reversibly fused trochanter and coxa (Truby 1985).

The antennae can also regenerate (Penzlin 1963; Urvoy 1963; Schafer 1973). At the first post-operative molt, the regenerate is composed of at least the first three articles

(Urvoy 1963), and the number of flagellar segments increases with subsequent molts (Schafer 1973).

Palps and cerci also regenerate (Penzlin 1963; Urvoy & Les Bris 1968). Following the first post-operative molt, the new appendage is often merely a bud or has reduced segmentation. The full number of segments is obtained after three to five molts, depending on the level of amputation and the time it is performed within the intermolt period (Urvoy & Les Bris 1968).

2.4.7 Isoptera

There is only indirect evidence of a PBP between trochanter and femur (Myles 1986).

It seems that legs can regenerate and attain full size, probably in approximately three molts (Myles 1986).

2.4.8 Mantodea

In this group (Bordage 1905), there is a PBP at the joint, marked externally by a furrow, between trochanter and femur, which are largely fused together.

Studies on autotomy and regeneration were carried out by Bordage (1905) on several species including *Mantis religiosa* and *M. prasina* (= *Paramantis prasina*). At least in these species, only meso- and metathoracic legs autotomize. Autotomy is produced by the strong extensor muscle that crosses the PBP. By contracting, it retracts in part into the trochanter. At the level of the PBP, there is no internal diaphragm. As a result, after autotomy, hemorrhage is only partly avoided by obstructing muscle fibers.

Regeneration following autotomy is usually fast and the legs are fully functional after the first molt. The regenerated leg, always with tetramerous tarsus, is usually smaller and slightly lighter than the undamaged leg (with pentamerous tarsus), but apparently does not differ from the former in ornamentation. Malformations are very rare, besides incomplete subdivision of the tarsus. Amputation distal to two thirds of the femur triggers appendotomy. Cuts in the last two tarsomeres do not produce any regenerate.

2.4.9 Orthoptera and Dermaptera

Legs have a PBP between trochanter and femur (Bordage 1905; Brousse-Gaury 1958). It is not crossed by muscles and a diaphragm prevents loss of hemolymph (reported for crickets by Graber as early as 1874).

According to Bordage (1905), only the jumping legs autotomize. However, in *Acheta*, autotomy occurs just more frequent in jumping legs than in the other legs (Brousse-Gaury 1958), and in *Scudderia texensis*, autotomily was found in the first two pairs of legs and autospasy in the jumping legs (Dixon 1989).

The regenerative power varies considerably within the orthopterans. In general, according to Lakes & Mücke (1989), the power of regeneration decreases within the Orthoptera, from crickets to tettigoniids to locusts. It is very low in *Ephippiger ephippiger* (Lakes & Mücke 1989). Interestingly, in newly hatched specimens, the amputation of the forelegs at the joint between femur and tibia leads to slow regeneration extending through all six instars. Eventually, a dimerous or trimerous regenerate is produced, composed of a tibial segment, a tarsal pad and the terminal claws. This regenerate is also reduced in size (just one quarter of normal length), sensory structures and neuronal pathways. In *Gryllus domesticus* (= *Achaeta domesticus*), the growth rate of the regenerating appendage is higher

than in undisturbed development, and there is complete regeneration of the leg up to the tarsus, independent of the level of amputation. However, the level of amputation does affect the size of the first regenerate and the number of regenerated spines, which, in any case, is lower than in the undamaged legs (Rościszewska & Urvoy 1989b). The number of spines and the spatial distribution of external sensory organs require a higher number of molts to reach full condition. In *Teleogryllus commodus*, regeneration is complete in gross morphology, but does not re-establish the full pattern of sensory structures such as subgenual and tympanal organs, and campaniform sensilla (Biggin 1981). According to Bordage (1905), in gryllids and in tettigoniids, the regenerate lacks the tympanal organ, but Huber (1987) reported complete regeneration of the tympanal organ in *Gryllus bimaculatus*, if amputation occurs at or distal to the femur-tibia joint.

In several tettigoniids, acridids and gryllids studied by Bordage (1905), the jumping legs never regenerated except for the tarsus. However, in the first two pairs of legs, extensive regeneration occurred if the specimen was young enough and the cuts separated trochanter from femur. By contrast, sectioning between coxa and trochanter resulted in a regenerate that was a more or less rudimentary stump. The tarsus, which was often lost during exuviation, always regenerated, although its growth was very slow and the resulting appendage was not completely functional (in general the tarsomeres were slightly different from those of an undamaged appendage). In the tettigoniids *Phylloptera laurifolia* and *Conocephalus differens* (= *Ruspolia differens*), the regenerate was tetramerous as the undamaged limb, in acridids and gryllids, trimerous as the undamaged limb (Bordage 1905). According to Chopard (1938), the regenerating tarsus never exceeds the number of four articles, irrespective of the number characteristic of the species (up to five articles). The first regenerate may still have a lower number of tarsal articles, and further articles are added during additional molts.

Chopard (1938) reported that regeneration of the orthopteran antenna is easily accomplished if part of the flagellum is removed, while it seems more difficult when the cut affects the two basal articles, which often results in anomalies, including heteromorphosis. The regenerated antenna is usually smaller, and in gryllids, individual articles are longer and different in shape. In *Acheta*, the size of regenerate and number of regenerated articles increase with subsequent molts (Rościszewska & Urvoy 1989a).

In earwigs, there is evidence of regeneration of the antennae (Przibram 1909; Chopard 1938).

2.4.10 *Phasmida*

Studies on autotomy and regeneration were carried out in *Bacillus rossius* by Godelmann (1901), in *Monandropoda inuncans*, *Raphiderus scabrosus* and *Eurycantha horrida* by Bordage (1905) and in *Carausius morosus* by Schindler (1979).

There is a PBP at the virtual (ankylosed) joint between trochanter and femur. Unlike the condition in cockroaches and mantids, no muscle crosses the PBP or is even inserted in the trochanter and there is a diaphragm crossed by nerves and tracheae only (Godelmann 1901; Bordage 1905).

Autotomy is triggered by excitation of the sensitive nerve of the leg. As in decapods, this process also occurs in beheaded animals. Sometimes autotomy does not work perfectly and the leg remains partly attached (Bordage 1905). Autotomy is very common in all instars, especially in nymphs after the third molt, but it can also occur in adults, though it is

much more difficult to trigger (Bordage 1905).

The regenerative power seems to be higher in young nymphs than in more aged ones and depends on the positional identity of the amputated limb: midlegs have the highest regenerative power, hindlegs the lowest (Godelmann 1901).

After the first post-operative molt, the leg regenerates with all final segments, even though it is usually reduced in size and ornamentation, and sometimes even ill-formed and not clearly segmented (Bordage 1905).

In *Bacillus rossius*, ablation of the last tarsomere or the last two tarsomeres does not trigger regeneration, while other cuts along the tarsus result in a regenerate of one or two segments. Amputations within the length of the first tarsomere cause the remaining tarsal stump to fall off, and the subsequent regeneration from the undamaged tibia produces a three- or four-segmented tarsus while the undamaged tarsus is five-segmented. Cuts at any level along the tarsus of the hindlegs are usually followed by appendotomy. Amputation in the distal part of the tibia leads either to appendotomy or to direct regeneration of a three- or four-segmented tarsus. Amputations along the femur always lead to autotomy. Regeneration from the PBP usually produces a four-segmented tarsus, but for amputations in the first instar a five-segmented tarsus was regenerated in seven cases out of 50. In one case, a nymph autotomized in the first instar regenerated at first a tarsus with four tarsomeres that changed into a five-segmented tarsus during the next molt (Godelmann 1901).

In *M. inuncans*, *R. scabrosus* and *Phyllium crurifolium*, the regenerated leg always has a four-segmented tarsus; cuts in the distal part of the tibia, or more distal to it, except for the last two tarsomeres, regenerate directly, while proximal cuts lead to autotomy. Interestingly, in *R. scabrosus*, amputation along the tibia produced a three-segmented tarsus that became four-segmented only after an additional molt (Bordage 1905).

If regeneration is induced from the distal part of the tibia, the femur also seems to contribute to the regenerative process since it becomes shorter and this shortening is not compensated for during subsequent molts (Godelmann 1901). Similarly, Bordage (1905) reported that coxa and trochanter both become shorter during regeneration following appendotomy. It is also noteworthy that regeneration from the PBP is faster than from any point distal to it (Bordage 1905).

The regenerative power of cerci and antennae is poor (Godelmann 1901). Urvoy (1970) carried out studies on regeneration of the antenna in *Sypyloidea sypylus*. Antennae were sectioned at various levels, using nymphs of various stages at different times during the intermolt. The regeneration potential decreased with age of the operated specimen, as indicated by smaller size and less numerous sensilla in the regenerate. Individual variation was observed, including differences between the two antennae of the same specimen. The nature of the regenerate depends on the level of sectioning. If it occurs proximal to the middle of the first antennal segment (the scape), there is no regenerate. If sectioning takes place between mid-scape and the articulation between second (pedicel) and third segment, the regenerate is a heteromorphic tarsus, more or less developed depending on the section level - the more distally the section is applied, the more developed is the appendage. In the heteromorphic appendage, the terminal part of the leg is always present, and the number of articles is fixed after the first molt. In some cases, there is a later increase in the size of the appendage and the number of sensilla. Finally, if the cut is distal to the proximal articulation of the third segment, the regenerate is an antenna in which the number of flagellar articles depends on age and level of the cut.

2.4.11 *Hemiptera*

A PBP has not been reported in this group.

Regeneration of lost appendages is poor and in several groups, especially in the homopterans, there is no regeneration at all. Experimental evidence, limited to a few heteropterans, showed that the regenerated leg is always reduced in size and often possesses an incomplete number of segments. No correlation with the nymphal stage at the time of operation has been noticed and, interestingly, the growth rate of the regenerating leg is not different from that of the opposite undamaged one (Lüscher 1948; Shaw & Bryant 1974).

There seems to be no regeneration following amputation proximal to the femur-tibia articulation. What remains of an amputated segment can be lost, e.g., following amputation at some level along the femur in the reduviid *Rhodnius prolixus*. In this species, the occasional appearance of a new, small terminal segment has been reported (Lüscher 1948). In the lygaeid *Oncopeltus fasciatus*, amputation in the middle of the femur results in a longer and deformed femur, sometimes followed by a new, small terminal segment (Shaw & Bryant 1974). Amputations at the femur-tibia articulation can result in a regenerative process or follow the same pattern as proximal cuts (Lüscher 1948; Shaw & Bryant 1974).

More distal amputations always start a regeneration process producing a leg with reduced segmentation. The final result depends on the level of the amputation and the number of molts ahead, since the regenerating leg will slowly increase the number of segments from instar to instar. In *R. prolixus* and *O. fasciatus*, where the number of tarsal segments should increase from two to three during the last molt, the final (adult) regenerate is sometimes a 'nymphal leg', i.e., one with two tarsomeres only. Regenerating legs have been documented following early amputation (before the first nymphal molt) in the middle of the tibia or more distal to it (Lüscher 1948; Shaw & Bryant 1974). In these cases, the number of tarsomeres increases from two to three during the last molt, as in an undamaged leg.

Cutting off the three distal segments of the antenna of *O. fasciatus* (the undamaged antenna has four segments) results in the regeneration of one segment, sometimes two. When regeneration is limited to one segment, the original bristle patterns of the two distal segments of the antenna is very often maintained. Sometimes, the regenerate is incompletely divided in two segments, and a complete division will not be attained during the remaining molts. In the regenerated antenna, the segments (including those left after amputation) usually become thicker and longer than expected (Shaw & Bryant 1974). The same was reported in the pentatomids *Raphigaster nebulosa* and *Euchistus variolarius* and several lygaeids (Wolsky 1957 and references therein). Removal of the two terminal segments of the antenna was followed by regeneration of only one segment (Wolsky 1957).

2.4.12 *Endopterygota*

There is no evidence that the larvae of holometabolans possess a PBP.

Evidence of regeneration is limited and the insect's response to the loss or breakage of appendages is far from uniform even within one order, as in Coleoptera. In this order, for example, regeneration of larval legs has been recorded in the tenebrionid *Tenebrio molitor*, the dynastid *Oryctes nasicornis*, the cerambycid *Rhagium indagator* (= *Hargium inquisitor*) (Megušar 1907), and to a limited extent also in the chrysomelid *Leptinotarsa decemlineata*, but in this case only following amputation in the first larval instar (Patay 1937; Poisson & Patay 1938). Lack of regeneration, however, has been reported for species of hydrophilids and dytiscids (Megušar 1907), and also in the chrysomelid *Timarcha* (Abeloos 1933; Bour-

don 1937).

The larval antennae of the moth *Lymantria dispar* regenerate their three articles passing through a stage of unsegmented bud (Kopeć 1913).

3 EVO-DEVO PERSPECTIVES ON ARTHROPOD APPENDAGE REGENERATION

3.1 Mechanisms

Before addressing specific questions about the diversity of regeneration processes in arthropod appendages and its possible evolutionary significance, it seems worthwhile to ask whether the comparative data summarized in the previous pages can help understanding regeneration and its mechanisms in more general terms.

The idea that regeneration is but a copy of ‘normal’ development has often been raised (e.g., Przibram 1909; Needham 1965). Taken literally, this idea is at the best grossly naive, nevertheless, it might be worth reconsidering, to some limited extent. We must first qualify this concept, however, since what we might call ‘normal’ development refers to two different processes, the equivalence of which is a matter for further speculation. In the vast majority of arthropods (a peculiar exception are polyembryonic wasps), normal development means embryogenesis, but in other metazoans, instead of embryogenesis, - or in addition to it - it could mean blastogenesis. Indeed, several authors (e.g., Dehorne 1916; Berrill 1952; Herlant-Meewis 1953) highlighted asexual reproduction, when comparing regeneration with ‘normal’ development. This is obviously relevant for groups such as annelids, for which this comparison has recently been revived. Bely & Wray (2001) interpreted fission as a derived condition from pre-existing regeneration mechanisms recruited for a new role. For arthropods, that lack comparable reproductive mechanisms, we could adopt a speculation suggested by Sánchez Alvarado (2000). This author proposed that embryonic limb buds might be phylogenetically derived from the same, locally acting ‘genetic organization’ originally deployed in the regeneration of damaged body parts. This idea comes close to Minelli’s (2000) notion of axis paramorphism, according to which the appendages are evolutionarily divergent copies of the main body axis.

When comparing regeneration to undisturbed developmental processes, one may be tempted to distinguish between adaptive regeneration following autotomy and regeneration as a general property of multicellular organisms. Such a clear-cut distinction, however, is unwarranted. Regeneration for certain traits has likely been shaped by natural selection. These include sophisticated mechanisms of autotomy with specialized autotomizer muscles and PBPs with protecting diaphragms and efficient re-arrangement of muscles, blood spaces and, when present, tracheae (e.g. crabs, cockroaches). In the case of the fiddler crabs, regeneration following autotomy has even become a developmental mechanism required to break up the initial body symmetry, which allows one of the male chelipeds to grow to its characteristic enormous size. In other cases, however, regeneration fails to produce a fully functional appendage, suggesting simple reactivation of growth and morphogenetic processes along unspecific pathways. For example, a dragonfly nymph may starve to death during the slow regeneration of the mask.

The fact that this local growth triggered by the loss of the appendage gives rise to a patterned regenerate rather than to a shapeless clump of cells has nourished the widespread

belief in the existence of a directed program. However, we should keep in mind that the regenerate is not produced in a vacuum of gene expression, but in a spatially and temporally well-defined and information-rich setting. In this context, it may be significant to note that regenerating a lost small, apical part of an appendage may be much more difficult than regenerating a whole appendage from a proximal PBP, in terms of time (or molts) required to obtain the regenerate as well as the completeness of the latter. In some decapods at least, vastly different mechanisms seem to be at work in the two cases, possibly due either to different availability of metabolic supply, or to different positional information, or to both.

3.1.1 *Regeneration... of what?*

Defective regeneration shows how much morphogenesis depends on epigenetic interactions (*sensu* Schlichting & Pigliucci 1998) between nerves, muscles, tracheae and epidermis during undisturbed growth and differentiation of arthropod appendages. During regeneration, nerves, muscles and tracheae often seem to not keep pace with the rapidly growing and differentiating epidermis (but sometimes, as in the hemipteran antenna, it seems to be the other way around). This was remarked as early as 1903 by Child and Young in their study of regenerating legs in damselflies and is reflected in the frequent defects found in the arrangement of sensilla along regenerated appendages.

We should perhaps say that the regeneration of an arthropod appendage is not the product of a distinct 'modular' process. In terms of mechanisms, it would be more appropriate to distinguish between regeneration of epidermis and cuticle, regeneration of nerves, regeneration of tracheae, and regeneration of muscles. The extent to which these processes are actually synchronized, eventually coupled together and limiting each other, will obviously determine the anatomical and functional quality of the regenerate. However, the latter should not be regarded as the product of a 'local program' or even of the 'local re-deployment of a limb-producing program', because such programs probably do not exist.

The common occurrence of a mismatch between the segmentation of the new appendage and the patterning of the sensilla along its proximo-distal axis also indicates independence of developmental events during the regeneration of the same appendage. A good example is the regenerated antenna of the isopod *Idotea*, which, following total ablation (a small part of the head also included), consists of epidermis and cuticle only, without any nerve or muscle (Bossuat 1958).

Quite often, the regenerate obtains the full number of segments, but these have an irregular set of sensilla (e.g., on the palps of the silverfish *Thermobia*; Larink 1983). In other cases, a more or less complete number of sensilla are developed on the regenerated appendage. However, when the regenerate is incompletely segmented, one 'double' segment can bear all the sensilla usually distributed over two segments. We discussed an example of such a condition in the section on the regenerating antenna of heteropterans. It is also similar to the regular condition in the antennule of adult males of some calanoid copepods (Boxshall & Huys 1998), and in the antennule of the isopod genus *Mancasellus* (= *Lirceus*) (Racovitza 1925).

3.1.2 *Local growth and competition*

The study of regeneration of arthropod appendages suggests that an organism is, to some extent, a mosaic of independent developmental domains (Paulian 1938). In other terms, growth and patterning of a regenerating appendage are largely autonomous from the re-

maining body. However, the regenerating appendage must compete for resources (Klingenberg & Nijhout 1998; Nijhout & Emlen 1998). The local rate of growth during regeneration can be exceptionally high, to the extent that the neighboring appendages are negatively affected by it (Hopkins 1985). Not surprisingly, therefore, production of a regenerate is sometimes accompanied by regression in size of segments of the appendage proximal to the level of the cut, as mentioned above for some species of stick insects. In decapod crustaceans, strong regression of ganglia and muscles proximal to the PBP is also common.

The convergence of the regenerate in size and complexity with the opposite appendage is extensive, and not easy to explain only in terms of competition for a shared pool of resources. In addition, this convergence is certainly not an absolute rule, as shown by the opposite (divergent) trend observed in heterochelous decapods such as *Uca*. In fiddler crabs, the loss of an appendage becomes a signal for a break of symmetry, starting a special allometric pathway of appendage growth.

3.1.3 *Regeneration, cuticle and mitosis*

Growth and differentiation are both involved in regenerating an appendage. Minimum requirements for growth are the detachment of the epithelium from the cuticle in a region proximal to the cut or the PBP, the recruitment of additional cells that will eventually result in a local blastema and the activation of mitosis therein. In mites other than ticks, lack of regeneration correlates positively with the lack of post-embryonic mitoses, and a similar explanation does perhaps apply to other small arthropods such as copepods, which apparently have very poor regeneration capability. Current evidence, however, does not cover a sufficient range of taxa to allow further speculations.

The detachment of the epidermis from the cuticle is probably required for morphogenesis no less than for cell proliferation. Similar to apolysis during the molting cycle, detaching the epidermal cells from the cuticle may release their mitotic potential, and, in addition, releases them from the morphostatic role of the cuticle (Minelli 2003b).

Generally speaking, regeneration is more effective in arthropods with higher and/or indeterminate number of molts than in those with a tight post-embryonic developmental schedule including a small, fixed number of molts: for example, regeneration is more conspicuous in isopods than in copepods, in cockroaches than in hemipterans. This point would require more systematic investigation.

3.1.4 *Appendotomy and regeneration*

The most efficient performances in regeneration are generally a follow-up to appendotomy, in particular autotomy. The two processes, however, are not necessarily interconnected. Most conspicuous is the lack of regeneration following autotomy of the jumping legs in the Orthoptera. This is surprising because the chances of survival (not to mention reproduction) of an autotomized specimen are likely to be dramatically small. The lack of regeneration of the appendotomized legs of some spiders and harvestmen is probably of lesser consequence for the fitness of the animal, if appendotomy is limited to one or a few legs.

3.2 Segmentation

3.2.1 Polarity of segment differentiation

In the new appendage, the tip is usually formed first. In scorpions, regeneration usually does not proceed further than the pretarsus. In this case, the tip of the appendage seems to generate a morphogenetic control over more proximal, original segments. For example, spine-like hairs typical of basitarsus and tarsus do appear on conserved pretarsal segments following amputation of the whole tarsal section, or more (Rosin 1964).

Unfortunately, evidence is poor as to the temporal order with which segmentation progresses in the regenerating appendage: literature data are often unreliable and sometimes conflicting, e.g., for decapod crustaceans. It seems safe to say, however, that in non-flagellar appendages segmentation proceeds neither in proximal to distal nor in distal to proximal sequence. The same was found by Norbeck & Denburg (1991) in the embryonic development in *Periplaneta*. For example, in the spider *Coelotes terrestris*, the regenerating equivalent to four distal-most leg articles (patella, tibia, metatarsus, tarsus) is at first represented by one segment, which later splits into two segments. Subsequently, the proximal segment is subdivided into patella and tibia, the distal segment into metatarsus and tarsus. This sequence agrees with the temporal sequence in undisturbed development (Vachon 1967).

3.2.2 Post-embryonic developmental schedules and regeneration: appendages with 'true segments' and 'flagella'

Post-embryonic development varies widely within arthropods, and a comparison between developmental schedules and regeneration processes suggests intriguing relationships.

Segmentation of the main body axis can follow two different modes. In epimorphic development, all body segments are already present at the end of embryonic development, whereas in anamorphic development juveniles hatch with an incomplete complement of segments. In the latter case, the final adult number of segments is reached later in ontogeny through a specific schedule of post-embryonic segment addition (Enghoff et al. 1993). Similarly, full segmentation of the developing appendages can be complete at their first appearance, or only later in ontogeny.

In the regeneration of appendages, the definitive number of segments in the regenerate (sometimes lower than the full number) is often complete within the first post-operative molt (e.g., in the legs of cockroaches and the spider *Dolomedes*). However, sometimes the number of segments increases according to an 'anamorphic' schedule (e.g., in the legs of zygopteran dragonflies). To some extent at least, the 'anamorphic' versus 'epimorphic' mode of regeneration matches the developmental mode, especially when comparing antennal development with antennal regeneration. The increase in the number of segments in the regenerating appendage recorded by Vachon (1956, 1967) for the spider *Coelotes* might seem an exception. However, although spiders are generally classified as epimorphic arthropods, their first free stage is embryo-like, and its appendages are still incompletely segmented. It was exactly this kind of 'larva' that Vachon investigated.

When different appendages of an arthropod or different parts of the same appendage can be contrasted as "truly segmental" versus "flagellar", their behavior during regeneration is generally also distinctly different. The number of flagellar units in the regenerate increases from molt to molt, whereas all 'true segments' are usually formed as soon as the regenerate emerges first. In appendages with flagellar organization, the number of flagellar units in the

regenerate is variable around a modal value that is mostly lower than in the undamaged appendage, but sometimes higher. An example is the flagellar tibia and tarsus of the whip-like forelegs of the Amblypygi.

3.3 Serial homology

What we know about regeneration of appendages in arthropods is largely based on legs and, to a much lesser extent, on antennae. However, whenever studies have been carried out, evidence of regeneration has been found for most kinds of appendages. For example, some spiders regenerate legs, palps, chelicerae, the labium and even the spinnerets (Bonnet 1930; Mikulska et al. 1975) and some decapods regenerate eyes and their stalks, gills, copulatory organs and uropods (Vernet & Charmantier-Daures 1994).

Evidence for the mouthparts of arthropods other than chelicerates is very limited. It has been claimed (von Buddenbrock 1954) that those of decapod crustaceans do not regenerate at all. As for insects, evidence is limited to palps and the mask of dragonfly nymphs.

Experiments on both antennae and legs of the same species (and possibly by the same author) are rare. Available studies do not show major differences in regenerative behavior between legs and other appendages: i.e., they either show regeneration or no regeneration at all.

Mechanism of appendotomy and regenerative power, however, are not necessarily uniform even among similar appendages of the same animal, e.g. among the thoracic legs of an insect.

Differences in regeneration power do not correlate with the degree of specialization of the appendage: in crabs, chelipeds and walking legs may exhibit similar performances in regeneration, while the three pairs of legs of stick insects perform differently despite their broadly similar morphology. Mechanisms of appendotomy, however, are often different in appendages with different specialization, for example jumping versus walking legs in orthopterans or raptorial versus walking legs in mantises. However, while in orthopterans the walking legs do not autotomize, the jumping legs do. In mantises, on the other hand, the specialized raptorial legs do not autotomize, while the walking legs do. We wonder whether this difference reflects a plesiomorphic positional gradient of increasing autotomy from fore to mid to hindlegs rather than the divergent adaptive specializations of these appendages.

3.4 Phylogenetic patterns

Due to the limited and irregularly scattered taxonomic sampling of our current database on autotomy and regeneration of arthropod appendages, it is not yet possible to study these phenomena using standard phylogenetic methods. There is abundant evidence that regeneration performances are sometimes very different even among closely related arthropods. The best example of this variability are probably the beetles: larval legs, as mentioned before, do not seem to regenerate in Hydrophilidae and Dytiscidae, but regenerate in other families.

Nevertheless, the lack of PBP and thus of any kind of appendotomy in millipedes, xiphosurans, scorpions, and mites (with the possible exception of *Opilioacarus*, which

represents a rather isolated clade within this group; cf. Vitzthum 1943; Brignoli 1967) is perhaps of phylogenetic significance. Some spiders also lack a PBP. In other spiders, the position of the PBP is variable (and there are even species that seem to possess more than one PBP). In centipedes the PBP is between the coxa and the trochanter. It seems significant that all the mentioned groups (a clade of chelicerates with myriapods, the Myriochelata of Pisani et al. 2004) lack real autotomy. Even the house centipede *Scutigera*, the only myriapod that loses its obviously fragile legs fairly easily in nature, seems to have auto-spasy or autotilly at most.

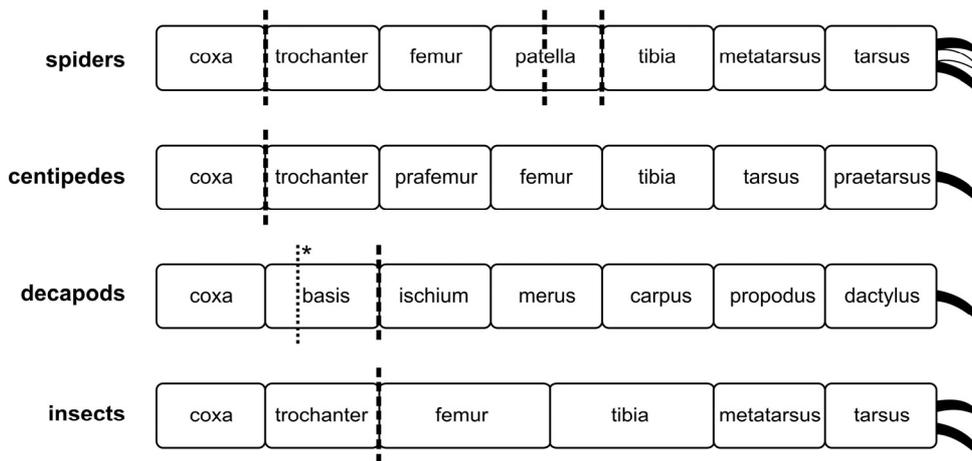


Figure 1. Position of the PBP along the leg in representatives of the major arthropod lineages. The appendages are drawn in very schematic and simplified way; in particular, possible secondary divisions of primary articles, e.g. in the tarsus, are ignored. The asterisk (*) marks the position of the PBP in the proximal part of the basis in isopods. Conditions in spiders are variable (see text).

The lack of PBP in the crustaceans other than malacostracans is probably also of phylogenetic significance. Only the remaining groups, malacostracans and hexapods, at least sometimes show real autotomy. The position of the PBP along the appendage is a further argument in favor of a Myriochelata/Pancrustacea hypothesis of higher relationships within the Arthropoda (Fig. 1), although caution is needed, owing to the incomplete data set available at the moment. Our comparison gives indirect support to Walossek & Müller's hypothesis (1997), that the most proximal articles of chelicerate (and trilobite) limbs are not homologous to the 'true' coxae of crustaceans. The PBP in insects seems to correlate well with the basis/ischium location in malacostracan crustaceans. Alternative locations of the PBP are probably a derived feature, especially when the PBP is not between two segments of the appendage, but within a segment, for example within the basis of the isopod leg. Another trait supporting a close relationship between malacostracans and hexapods is the presence of a PBP with a diaphragm that is not crossed by muscles. This condition has probably been lost at least twice in insect evolution. The Dictyoptera have retained a trochantero/femoral PBP, but apparently lost the diaphragm. In the Hemipteroidea and the (larval) Endopterygota, a true PBP has disappeared. On the other hand, a PBP with dia-

phragm, in a probably not-homologous position, has evolved in *Scutigera* within the Myriochelata. This is certainly not the only character in *Scutigera* that presents a convergence with insects.

ACKNOWLEDGEMENTS

We are grateful to many friends and colleagues for their advice on appendage regeneration in different arthropod groups, to Stefan Koenemann and two anonymous referees for their precious comments on a previous draft of this paper and to Ariel Chipman for a careful linguistic check of the final manuscript. We thank also Angela Mazzon, Monica Ortolan and Donata Pieri (Vallisneri Library, University of Padova) for their generous assistance with our extensive literature.

REFERENCES

- Abeloos, M. 1933. Sur la régénération des pattes chez le coléoptère *Timarcha violaceo-nigra* De Geer. *C.R. hebdom. Séanc. Acad. Sci. Paris* 113: 17-19.
- Adiyodi, R.G. 1972. Wound healing and regeneration in the crab *Paratelphusa hydrodromous* [sic]. *Int. Rev. Cyt.* 32: 257-285.
- Agar, W.E. 1930. A statistical study of regeneration in two species of Crustacea. *J. Exp. Biol.* 7: 349-369.
- Ameer Hamsa, K.M.S. 1982. Observations [sic!] on moulting of crab *Portunus pelagicus* Linnaeus reared in the laboratory. *J. Mar. Biol. Ass. India* 24: 69-71.
- Bain, B.A. 2003. Postembryonic development in the pycnogonid *Austropallene cornigera*. *Invertebr. Repr. Dev.* 43: 181-192.
- Bateson, W. 1894. *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species*. London: Macmillan.
- Belozerov, V.N. 2001. Regeneration of limbs and sensory organs in ixodid ticks (Acari, Ixodoidea, Ixodidae and Argasidae). *Russian J. Dev. Biol.* 32: 129-142.
- Bely, A.E. & Wray, G.A. 2001. Evolution of regeneration and fission in annelids: insights from *en-grailed-* and *orthodenticle-*class gene expression. *Development* 128: 2781-2791.
- Berrill, N.J. 1952. Regeneration and budding in worms. *Biol. Rev.* 27: 401-438.
- Biggin, R.J. 1981. Pattern re-establishment - transplantation and regeneration of the leg in the cricket *Teleogryllus commodus* (Walker). *J. Embryol. Exp. Morphol.* 61: 87-101.
- Bliss, D.E. 1960. Autotomy and regeneration. In: Waterman, T.H. (ed.), *The Physiology of Crustacea*, 1: 561-589. New York: Academy Press.
- Bonnet, P. 1930. La mue, l'autotomie et la régénération chez les araignées avec une étude des Dolomès d'Europe. *Bull. Soc. Hist. Nat. Toulouse* 59: 237-700.
- Bordage, E. 1905. Recherches anatomiques et biologiques sur l'anatomie et régénération chez diverses arthropodes. *Bull. Sci. France Belgique* 39: 307-454.
- Bossuat, M. 1958. Territoire de régénération des antennes de l'isopode *Idotea baltica* (Aud.). *C.R. hebdom. Séanc. Acad. Sci. Paris* 246: 2530-2532.
- Bourdon, J. 1937. Sur la régénération des ébauches de quelques organes imaginaires chez le coléoptère *Timarcha goettingensis* L. *C.R. hebdom. Séanc. Acad. Sci. Paris* 124: 872-874.

- Boxshall, G.A. & Huys, R. 1998 The ontogeny and phylogeny of copepod antennules. *Phil. Trans. R. Soc. London, Ser. B*, 353: 765-786.
- Brignoli, M.P. 1967. Su *Opilioacarus italicus* (With). *Fragm. Entomol.* 5: 111-121.
- Brousse-Gaury, P. 1958. Contribution a l'étude de l'autotomie chez *Acheta domestica* L. *Bull. Sci. France Belgique* 92: 55-85.
- Bullière, D. & Bullière, F. 1985. Regeneration. In: Kerkut, G.A. & Gilbert, L.I. (eds.), *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, 2: 371-424. Oxford: Pergamon Press.
- Calman, W.T. 1909. *A Treatise on Zoölogy, Part 7, Appendiculata, 3rd Fascicle, Crustacea*. London: Adam & Charles Black.
- Cameron, J.A. 1926. Regeneration in *Scutigera forceps*. *J. Exp. Zool.* 46: 169-179.
- Charniaux-Cotton, H. 1957. Croissance, régénération et déterminisme endocrinien des caractères sexuels d'*Orchestia gammarella* (Pallas) crustacé amphipode. *Ann. Sci. Nat. Zool. Biol. Anim.* 19: 413-559.
- Child, C.M. & Young, A.N. 1903. Regeneration of appendages in nymphs of the Agrionidae. *Arch. Entwicklungsmech. Organ.* 15: 543-602.
- Chopard, L. 1938. *La Biologie des Orthoptères*. Paris: Paul Lechevalier.
- Clare, A., Lumb, G., Clare, P.A. & Costolow, J.D. 1990. A morphological study of wound response and telson regeneration in postlarval *Limulus polyphemus* (L.). *Invertebr. Repr. Dev.* 17: 77-87.
- Cloudsley-Thompson, J.L. 1961. A new sound-producing mechanism in centipedes. *Entomol. Monthly Mag.* 96: 110-113.
- Coineau, Y. & Legendre, R. 1975. Sur un mode de régénération che les Arthropodes: la régénération des pattes marcheuses che les Opilioacarines (Acari: Notostigmata). *C.R. hebdom. Séanc. Acad. Sci. Paris, Sér. D, Sci. Nat.*, 280: 41-43.
- Condé, B. 1955. Matériaux pour une monographie des Diploures Campodéidés. *Mém. Mus. Nat. Hist. Nat. Paris, Sér. A: Zool.*, 12: 1-201.
- Darwin, C. 1854. *A monograph on the sub-class Cirripedia*. London: Ray Society.
- Degrange, C. & Seassau, M. 1974. Sur la régénération du masque (= labium) des larves des odonates anisoptères *Anax imperator* Leach et *Aeschna cyanea* (Müller). *C.R. hebdom. Séanc. Acad. Sci. Paris, Sér. D: Sci. Nat.*, 278: 281-284.
- Dehorne, L. 1916. Les naïdimorphes et leur reproduction asexuée. *Arch. Zool. Exp. Gén.* 56: 25-157.
- Desai, U.M. & Achuthankutty, C.T. 2000. Complete regeneration of ablated eyestalk in penaeid prawn, *Penaeus monodon*. *Curr. Sci.* 79: 1602-1603.
- Dixey, L. 1938. Effects of repeated regeneration of the antenna in *Gammarus chevreuxi*. *Proc. Zool. Soc. London* 108: 289-296.
- Dixon, K.A. 1989. Effect of leg type and sex on autotomy in the Texas bush katydid, *Scudderia texensis*. *Can. J. Zool.* 67: 1607-1609.
- Dohrn, A. 1881. Die Pantopoden des Golfes von Neapel. *Fauna und Flora des Golfes von Neapel, Monographie* 3.
- Edwards, J.S. 1972. Limb loss and regeneration in two crabs: the king crab *Paralithodes camtschatica* and the tanner crab *Chionoecetes bairdi*. *Acta Zool.* 53: 105-112.
- Enghoff, H., Dohle, W. & Blower, J.G. 1993. Anamorphosis in millipedes (Diplopoda). The present state of knowledge and phylogenetic considerations. *Zool. J. Linn. Soc.* 109: 103-234.
- Ernsting, G. & Fokkema, D.S. 1983. Antennal damage and regeneration in springtails (Collembola) in relation to predation. *Netherl. J. Zool.* 33 (4): 476-484.
- Gaubert, P. 1892. Autotomie chez les Pycnogonides. *Bull. Soc. Zool. France* 17: 224-225.

- Godelmann, R. 1901. Beiträge zur Kenntnis von *Bacillus Rossii* Fabr. Mit besonderer Berücksichtigung der bei ihm vorkommenden Autotomie und Regeneration einzelner Gliedmassen. *Arch. Entwicklungsmech. Organ.* 12: 265-301.
- Govind, C.K. & Read, A.T. 1994. Regenerate limb bud sufficient for claw reversal in adult snapping shrimps. *Biol. Bull.* 186: 241-246.
- Graber, V. 1874. Ueber eine Art fibrilloiden Bindengewebes der Insektenhaut und seine lokale Bedeutung als Tracheensuspensorium. *Arch. Mikrosk. Anat.* 10.
- Hartnoll, R.G. 1988. Growth and molting. In: Burgreen, W.W. & McMahon, B.R. (eds.), *Biology of Land Crabs*: 186-210. Cambridge: Cambridge Univ. Press.
- Herbst, C. 1891. Beiträge zur Kenntnis der Chilopoden. *Bibl. Zool.* 3: 1-42.
- Herlant-Meewis, H. 1953. Contribution à l'étude de la régénération chez les oligochètes Aeolosomatidae. *Ann. Soc. R. Zool. Belg.* 84: 117-161.
- Hopkins, P.M. 1985. Regeneration and relative growth in the fiddler crab. *Crustacean Issues* 3: 265-275.
- Hopkins, P.M. 1993. Regeneration of the walking legs in the fiddler crab *Uca pugilator*. *Am. Zool.* 33: 348-356.
- Huber, F. 1987. Plasticity in the auditory system of crickets: phonotaxis with one ear and neuronal reorganization within the auditory pathway. *J. Comp. Physiol.* 161: 583-604.
- Igelmund, P. 1987. Morphology, sense organs, and regeneration of the forelegs (whips) of the whip spider *Heterophrynus elaphus* (Arachnida, Amblypygi). *J. Morphol.* 193: 75-89.
- Juanes, F. & Smith, D.L. 1995. The ecological consequences of limb damage and loss in decapod crustaceans: a review and prospectus. *J. Exp. Mar. Biol. Ecol.* 193: 197-223.
- Kaars, C., Greenblatt, S. & Fournier, C.R. 1984. Patterned regeneration of internal femoral structures in the cockroach, *Periplaneta americana* L. *J. Exp. Biol.* 230: 141-144.
- Kaestner, A. 1968. *Invertebrate Zoology*, 2. New York, London, Sidney: Interscience Publishers.
- Klepál, W. & Barnes, H. 1974. Regeneration of the penis in *Balanus balanoides* (L.). *J. Exp. Mar. Biol. Ecol.* 16: 205-211.
- Klingenberg, C.P. & Nijhout, H.F. 1998. Competition among growing organs and developmental control of morphological asymmetry. *Proc. Zool. Soc. London, Ser. B*, 265: 1135-1139.
- Knoflach, B. & van Harten, A. 2000. Palpal loss, single palp copulation and obligatory mate consumption in *Tidarren cuneolatum* (Tullgren, 1910) (Araneae, Theridiidae). *J. Nat. Hist.* 34: 1639-1659.
- Kocian, V. 1930. Un cas d'hétéromorphose chez *Argulus foliaceus* L. *Arch. Zool. Exp. Gén.* 70: 23-27.
- Kopeć, S. 1913. Untersuchungen über die Regeneration von Larvalorganen und Imaginalscheiben bei Schmetterlingen. *Arch. Entwicklungsmech. Organ.* 37: 440-472.
- Lakes, R. & Mücke, A. 1989. Regeneration of the foreleg tibia and tarsi of *Ephippiger ephippiger* (Orthoptera: Tettigoniidae). *J. Exp. Zool.* 250: 176-187.
- Larink, O. 1983. Regeneration des Sensillenmusters der Labialpalpen bei Lepismatidae (Insecta: Zygentoma). *Verh. Deutsch. Zool. Ges.* 1983: 302.
- Lawrence, R.F. 1953. *The Biology of the Cryptic Fauna of Forest, with Special Reference to the Indigenous Forest of South Africa*. Cape Town, Amsterdam: A.A. Balkema.
- Lewis, J.G.E. 1968. Individual variations in a population of the centipede *Scolopendra amazonica* from Nigeria and its implications for methods of taxonomic discrimination in Scolopendridae. *J. Linn. Soc. London, Zool.*, 47: 315-326.

- Lewis, J.G.E. 2000. Centipede antennal characters in taxonomy with particular reference to scolopendromorphs and antennal development in pleurostigmomorphs (Myriapoda, Chilopoda). In: Wytwer, J. & Golovatch, S. (eds.), *Progress in Studies on Myriapoda and Onychophora. Fragm. Faun. Warszawa* 43 (suppl.): 87-96.
- Lüscher, M. 1948. The regeneration of legs in *Rhodnius prolixus* (Hemiptera). *J. Exp. Biol.* 25: 334-343.
- Mariappan, P., Balasundaram, C. & Schmitz, B. 2000. Decapod crustacean chelipeds: an overview. *J. Bioscience* 25: 301-313.
- McConaughy, J.R. 1991. Limb regeneration in juveniles of the mud crab *Rhithropanopeus harrisi* following removal of developing limb buds. *J. Exp. Zool.* 257: 64-69.
- Megušar, F. 1907. Die Regeneration der Koleopteren. *Arch. Entwicklungsmech. Organ.* 26: 148-234.
- Mellon, D. & Tewari, M.K. 2000. Heteromorphic antennules protect the olfactory midbrain from atrophy following chronic antennular ablation in freshwater crayfish. *J. Exp. Zool.* 286: 90-96.
- Mikulska, I., Jacuński, L. & Weychert, K. 1975. The regeneration of appendages in *Tegenaria artica* C.L. Koch (Agelenidae, Araneae). *Zool. Poloniae* 25: 99-109.
- Minelli, A. 2000. Limbs and tail as evolutionarily diverging duplicates of the main body axis. *Evol. Dev.* 2: 157-165.
- Minelli, A. 2003a. The origin and evolution of appendages. *Int. J. Dev. Biol.* 47: 573-581.
- Minelli, A. 2003b. *The Development of Animal Form*. Cambridge: Cambridge Univ. Press.
- Minelli, A., Foddai, D., Pereira, L.A. & Lewis, J.G.E. 2000. The evolution of segmentation of centipede trunk and appendages. *J. Zool. Syst. Evol. Res.* 38: 103-117.
- Morgan, T.H. 1920. Variations in the secondary sexual characters of the fiddler crab. *Am. Nat.* 54: 220-246.
- Morgan, T.H. 1923. The development of asymmetry in the fiddler crab. *Am. Nat.* 57: 269-273.
- Murakami, Y. 1958. The life history of *Bothropolys asperatus* Koch. *Zool. Mag. Tokyo* 67: 217-223 [in Japanese, with English summary].
- Mykles, D.L. 2001. Interactions between limb regeneration and molting in decapod crustaceans. *Am. Zool.* 41: 399-406.
- Myles, T.G. 1986. Evidence of parental and/or sibling manipulation in three species of termites in Hawaii (Isoptera). *Proc. Hawai. Entomol. Soc.* 27: 129-136.
- Needham, A.E. 1947. Local factors and regeneration in Crustacea. *J. Exp. Biol.* 24: 220-226.
- Needham, A.E. 1965. Regeneration in the Arthropoda and its endocrine control. In: Kiortsis, V. & Trampusch, H.A.L. (eds.), *Regeneration in Animals and Related Problems*: 283-323. Amsterdam: North-Holland Publ. Comp.
- Newport, G. 1844. On the reproduction of lost part in Myriapoda and Insecta. *Phil. Trans. R. Soc. London* 1844: 283-294.
- Nijhout, H.F. & Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. *Proc. Nat. Acad. Sci. Un. St.* 95: 3685-3689.
- Nilsson, C. 1986. The occurrence of lost and malformed legs in mayfly nymphs as a result of predator attacks. *Ann. Zool. Fenn.* 23: 57-60.
- Niwa, K. & Kurata, H. 1964. Limb loss and regeneration in the adult king crab *Paralithodes camtschatica*. *Bull. Hokkaido Reg. Fish. Res. Lab.* 28: 51-55.
- Norbeck, B.A. & Denburg, J.L. 1991. Pattern formation during insect leg segmentation: studies with a prepattern of a cell surface antigen. *Roux's Arch. Dev. Biol.* 199: 476-491.

- Noulin, G. 1962. Contribution à l'étude d'un plan de rupture et des mécanismes d'amputation des appendices chez les Oniscoïdes (Crustacés Isopodes). *87 Congrès des Sociétés Savantes, Limoges*: 1159-1163.
- Noulin, G. 1984. *Morphogenèse régénératrice chez les Crustacé Isopode Porcellio dilatatus: histologie et cytologie, interactions avec la mue, formations surnuméraires*. Thèse de Doctorat d'État, Université de Poitiers.
- Nouvel-van Rysselberge, L. 1937. Contribution à l'étude de la mue, de la croissance et de la régénération chez les Crustacés Natantia. *Rec. Inst. Zool. Torley-Rousseau, Bruxelles*, 6: 5-161.
- Patay, R. 1937. Sur la régénération des pattes chez le coléoptère chrysomélide *Leptinotarsa decemlineata* Say. *C.R. hebdom. Séanc. Acad. Sci. Paris, Sér. D, Sci. Nat.*, 126: 283-285.
- Paulian, R. 1938. Contribution à l'étude quantitative de la régénération chez les Arthropodes. *Proc. Zool. Soc. London, Ser. A*, 108: 297-383.
- Penzlin, H. 1963. Über die Regeneration bei Schaben (Blattaria). I. Das Regenerationsvermögen und die Genese des Regenerats. *W. Roux's Arch. Entwicklungsmech. Organ.* 154: 434-465.
- Pisani, D., Poling, L.L., Lyons-Weiler, M. & Hedges, S.B. 2004. The colonization of land by animals: molecular phylogeny and divergence times among arthropods. *BMC Biology* 2004, 2: 1. On-line available at: <http://www.biomedcentral.com/1741-7007/2/1>.
- Poisson, R. & Patay, R. 1938. Sur quelques modalités de la régénération des pattes et des ailes chez la larve du doryphore: *Leptinotarsa decemlineata* Say. *C.R. hebdom. Séanc. Acad. Sci. Paris* 129: 126-128.
- Przibram, H. 1909. *Regeneration*. Leipzig, Wien: Franz Deuticke.
- Przibram, H. & Werber, E.J. 1907. Regenerationsversuche allgemeiner Bedeutung bei Borstenschwänzen (Lepismatidae). *Arch. Entwicklungsmech. Organ.* 27: 615-631.
- Rabes, O. 1907. Regeneration der Schwanzfäden bei *Apus cancriformis*. *Zool. Anz.* 24: 753-755.
- Racovitza, E. 1925. Notes sur les Isopodes. 13. Morphologie et phylogénie des antennes II, Le fouet. *Arch. Zool. Exp. Gén.* 63: 533-622.
- Randall, J.B. 1981. Regeneration and autotomy exhibited by the black widow spider, *Latrodectus variolus* Walckenaer. *W. Roux's Arch. Dev. Biol.* 190: 230-232.
- Read, A.T. & Govind, C.K. 1997a. Regeneration and sex-biased transformation of the sexually dimorphic pincer claw in adult snapping shrimps. *J. Exp. Zool.* 279: 356-366.
- Read, A.T. & Govind, C.K. 1997b. Claw transformation and regeneration in adult snapping shrimp: test of the inhibition hypothesis for maintaining bilateral asymmetry. *Biol. Bull.* 193: 401-409.
- Rockett, C.L. & Woodring, J.P. 1972. Comparative studies of acarine limb regeneration, apolysis, and ecdysis. *J. Insect Physiol.* 18: 2319-2336.
- Rogers, D.C. 2001. Revision of the Nearctic *Lepidurus* (Notostraca). *J. Crust. Biol.* 21: 911-1006.
- Rościszewska, M. & Urvoy, J. 1989a. Contribution à l'étude de la régénération d'appendices chez *Gryllus domesticus* L. (Orthoptera), I, Étude de la régénération de l'antenne. *Acta Biol. Cracov., Ser. Zool.*, 31: 125-135.
- Rościszewska, M. & Urvoy, J. 1989b. Contribution à l'étude de la régénération d'appendices chez *Gryllus domesticus* L. (Orthoptera), II, Étude de la régénération des pattes. *Acta Biol. Cracov., Ser. Zool.*, 31: 136-143.
- Rosin, R. 1964. On regeneration in scorpions. *Israel J. Zool.* 13: 177-183.
- Roth, V.D. & Roth, M.R. 1984. A review of appendotomy in spiders and other arachnids. *Bull. Brit. Arachnol. Soc.* 6: 137-146.
- Sánchez Alvarado, A. 2000. Regeneration in the metazoans: why does it happen? *BioEssays* 22: 578-590.

- Schafer, R. 1973. Postembryonic development in the antenna of the cockroach, *Leucophaea maderae*: growth, regeneration and the development of the adult pattern of sense organs. *J. Exp. Zool.* 183: 353-364.
- Scheffel, H. 1987. Häutungsphysiologie der Chilopoden: Ergebnisse von Untersuchungen an *Lithobius forficatus* (L.). *Zool. Jahrb., Abt. Allgem. Zool. Physiol. Tiere*, 91: 257-282.
- Scheffel, H. 1989. Zur wechselseitigen Beeinflussung von Regeneration und Häutung bei Larven des Chilopoden *Lithobius forficatus* (L.). *Zool. Jahrb., Abt. Allgem. Zool. Physiol. Tiere*, 93: 436-505.
- Schindler, G. 1979. Funktionsmorphologische Untersuchungen zur Autotomie der Stabheuschrecke *Carausius morosus* Br. (Insecta: Phasmida). *Zool. Anz.* 303: 316-326.
- Schlichting, C.D. & Pigliucci, M. 1998. *Phenotypic evolution: a reaction-norm perspective*. Sunderland, Massachusetts: Sinauer.
- Shaw, V.K. & Bryant, P.J. 1974. Regeneration of appendages in the large milkweed bug, *Oncopeltus fasciatus*. *J. Insect Physiol.* 20: 1849-1857.
- Skinner, D.M. 1985. Molting and regeneration. In: Bliss, D.E. & Mantel, L.H. (eds.), *The Biology of Crustacea*, 9: 43-146. New York: Academic Press.
- Spivak, E.D. & Politis, M.A. 1989. High incidence of limb autotomy in a crab population from a coastal lagoon in the province of Buenos Aires, Argentina. *Can. J. Zool.* 67: 1976-1985.
- Sweetman, H.L. 1934. Regeneration of appendages and molting among the Thysanura. *Bull. Brooklyn Entomol. Soc.* 29: 158-161.
- Tanaka, A., Akahane, H. & Ban, Y. 1992. The problem of the number of tarsomeres in the regenerated cockroach leg. *J. Exp. Zool.* 262: 61-70.
- Toyota, K., Yamauchi, T. & Miyajima, T. 2003. A marking method of cutting uropods using malformed regeneration for kuruma prawn *Marsupenaeus japonicus*. *Fisheries Science*, 69, 161-169.
- Truby, P.R. 1985. Separation of wound healing from regeneration in the cockroach leg. *J. Embryol. Exp. Morphol.* 85: 177-190.
- Urvoy, J. 1963. Étude anatomo-fonctionnelle de la patte et de l'antenne de la blatte *Blabera craniifer* B. *Ann. Sci. Nat. Zool. Biol. Anim.* 12: 287-413.
- Urvoy, J. 1970. Étude des phénomènes de régénération après section d'antenne chez le phasme *Spyloidea sypylus* W. *J. Embryol. Exp. Morphol.* 23: 719-728.
- Urvoy, J. & Les Bris, R. 1968. Étude de la régénération des cerques, des palpes labiaux et des palpes maxillaires chez *Blabera craniifer* (Orth. Blattidae). *Ann. Soc. Entomol. France* 4: 371-383.
- Vachon, M. 1956. Remarques sur la morphogenèse au cours de la régénération des pattes chez les araignées. *Proceedings of the XIV International Congress of Zoology, Copenhagen, 1953*.
- Vachon, M. 1957. La régénération appendiculaire chez les scorpions (Arachnides). *C.R. hebdom. Séanc. Acad. Sci. Paris* 244: 2556-2559.
- Vachon, M. 1967. Nouvelles remarques sur la régénération des pattes chez l'araignée: *Coelotes terrestris* Wid. (Agelenidae). *Bull. Soc. Zool. France* 92: 417-428.
- Verhoeff, K.W. 1902-1925. Chilopoda. In: *H.G. Bronn's Klassen und Ordnungen des Tier-Reichs*, 5 (2): 1-725. Leipzig: C.F. Winter.
- Vernet, G. & Charmantier-Daures, M. 1994. Mue, autotomie et régénération. In: Grassé, P.P. (ed.), *Traité de Zoologie, Tome 7, Fascicule 1*: 153-194. Paris: Masson.
- Vitzthum, G. 1943. Acarina. In: *H. G. Bronn's Klassen und Ordnungen des Tier-Reichs, Bd. 5, Abt. 4, Buch 5*: 1-1011. Leipzig: C.F. Winter.
- Vollrath, F. 1990. Leg regeneration in web spider and its implications for orb spider exploration and web-building behaviour. *Bull. British Arachnol. Soc.* 8: 177-184.

- Vollrath, F. 1995. Lyriform organs on regenerated spider legs. *Bull. British Arachnol. Soc.* 10: 115-118.
- von Buddenbrock, W. 1954. Physiologie der Decapoden. Die Regeneration. In: *H.G. Bronn's Klassen und Ordnungen des Tier-Reichs, Bd. 5, Abt. 1, Buch 7, Lfg. 9*: 1231-1246. Leipzig: Akademische Verlagsgesellschaft.
- Walossek, D. & Müller, K.J. 1997. Cambrian 'Orsten'-type arthropods and the phylogeny of Crustacea. In: Fortey, R.A. & Thomas, R.H. (eds.), *Arthropod Relationships*: 139-153. London: Chapman & Hall.
- Wege, W. 1911. Morphologische und experimentelle Studien an *Asellus aquaticus*. *Zool. Jahrb., Abt. Allgem. Zool. Physiol. Tiere*, 30: 217-320.
- Weis, J.S. 1982. Studies on limb regeneration in the anomuran *Pagurus longicarpus* and *Emerita talpoidea*. *J. Crust. Biol.* 2: 227-231.
- Weise, R. 1991. Regeneration of antennae in the third larvae of *Lithobius forficatus*. *Zool. Anz.* 227: 343-355.
- Weygoldt, P. 1984. L'autotomie chez les Amblypyges. *Rev. Arachnol.* 5: 321-327.
- Wolsky, A. 1957. "Compensatory hyper-regeneration" in antennae of Hemiptera. *Nature* 180: 1144-1145.
- Wood, F.D. 1926. Autotomy in Arachnida. *J. Morphol. Physiol.* 42: 143-195.
- Wood, F.D. & Wood, H.E. 1932. Autotomy in decapod Crustacea. *J. Exp. Zool.* 62: 1-55.
- Wygodzinsky, P. 1941. Beiträge zur Kenntnis der Dipluren und Thysanuren der Schweiz. *Denkschr. Schweiz. Naturf. Ges.* 74: 113-227.
- Yamaguchi, T. 2001. Dimorphism of chelipeds in the fiddler crab, *Uca arcuata*. *Crustaceana* 74: 913-923.