The effect of amputation of polymelic walking legs and of parts of appendage complexes in *Tegenaria atrica* (Araneae: Agelenidae)

Teresa Napiórkowska, **Lech Jacuński** and **Julita Templin**
Department of Invertebrate Zoology,
Institute of General and Molecular Biology,
Nicolaus Copernicus University,
Gagarina 9, 87-100 Toruń, Poland

Summary

Experiments on the epimorphic capabilities of polymelic walking appendages and those forming so-called complex anomalies in *Tegenaria atrica* C. L. Koch were carried out in two stages. First the appropriate changes in the structure of the prosoma were induced using the teratogenic effect of alternating temperatures (14 and 32°C, changing every 12 h) on developing embryos. Secondly, some fragments of the deformed appendages were amputated using steelon thread ligatures in order to observe the successive phases of the regeneration process. Until the termination of the experiments, no external regenerates were found on the post-amputation surfaces in any of the operated individuals.

Introduction

In spiders, as in other arthropods belonging to various systematic taxa, there occur processes of epimorphic regeneration. This mode of regeneration can be defined in most general terms as the re-creation of a part of the body at the place of its loss or injury without the necessity of reorganising the whole organism (Jura & Krzanowska, 1998). In arthropods epimorphosis occurs mainly in the appendages, which owing to their jointed structure have become convenient material for comprehensive studies of these processes. It is possible to carry out comparative anatomical analysis of the developed regenerate and its sister appendage developing without disturbance. In the course of an experiment it is also possible to take measurements of the regenerated fragment of the leg by defining accurately the place of amputation. Moreover, the presence on the leg joints of characteristic external structures of cuticular origin, such as spines, setae and claws, makes it easier to assess the regenerative potential and all departures from normality. It is then possible to trace the characteristic succession of joint development in the course of epimorphosis and to establish the successive regeneration phases. Based on these properties of the appendages, Vachon (1967) distinguished four stages in the development of regenerates after traumatic epimorphosis in *Coelotes terrestris* (Wider) (Amaurobiidae), corresponding to four successive stages of their ontogenetic development. According to Vachon (1967) the appearance of a certain type of regenerate (embryonal, prelarval, larval, nymphal) depended directly on the time of amputation. Thorough studies of each regenerated joint made Vachon adopt the hypothesis that the regenerates were not homogeneous, since in *Coelotes terrestris* they consisted of joints of different ages, the most distal ones being the oldest. Similar results were obtained by Mikulska *et al.* (1975) and Jacuński *et al.* (1994) while

studying the epimorphic regeneration of appendages in the initial stages of postembryogeny in *Tegenaria atrica* C. L. Koch (Agelenidae).

Other arachnologists who have studied the capability for appendage regeneration include Friedrich (1906), Weiss (1907), Oppenheim (1908), Wood (1926), Gabritschevsky (1927, 1930), Bonnet (1930), Locket (1936), Savory (1936), Ruhland (1976), Randall (1981), Roth & Roth (1984) and Vollrath (1990). According to these authors epimorphosis of the feeding and walking appendages of the prosoma is a common phenomenon, and as long as the spider is capable of moulting it can regenerate its lost appendages. Moreover, it is capable of carrying out regenerative action repeatedly in its postembryonic life. Simultaneous re-creation of fragments of several appendages is also possible, irrespective of the location of amputation. The observations of these researchers were concerned mainly with appendages of normal anatomical structure, which had developed in undisturbed ontogeny. Therefore, the question arises whether polymelic appendages and those that are parts of complicated appendage complexes have similar epimorphic capabilities. Such anomalies can develop as a result of teratogenic factors acting on developing embryos.

One of these factors is temperature. Its teratogenic effect was noticed by Holm (1940), who described dichotomy of the prosoma in *Aranea sexpunctata* (L.) (=*Nuctenea umbratica* (Clerck)) and reduction of the opisthosoma in *Tapinopa longidens* (Wider), and Juberthie (1962, 1963a, b, 1968), who studied the effect of increased temperature on the embryonic development of Opiliones. Similarly, Jacuński (1969, 1971), Mikulska (1973) and Mikulska & Jacuński (1970, 1971) induced developmental monstrosities in *Argyroneta aquatica* (Clerck) and *Tegenaria atrica*. An even more effective teratogenic factor, giving surprising results, is alternating temperatures. The experimental use of this factor induced the development of anomalies in nearly all metameres in *T. atrica* (Jacuński, 1984, 2002; Jacuński *et al.*, 2004; Jacuński & Templin, 2003).

One of the morphological anomalies induced by exposure of *T. atrica* embryos to alternating temperatures is polymely, which consists of the development on the germ band of accessory metameres, either entire or as halves (right or left). This changes the overall metamery of the prosoma and results in the development of accessory appendages, i.e. appendages which are absent in normal ontogenic development. In the internal structure of polymelic individuals an increase in the volume of the nervous mass and changes in musculature can be observed (Jacuński *et al.*, 2002). Complex anomalies are another kind of anomaly which may develop under the same experimental conditions. Their characteristic feature is the overlapping of at least two different deformities of appendages. Either type of monstrosity can cause high mortality among spider populations, which has been confirmed by researchers' reports. It therefore seems important to find out what kind of mechanism has been developed to protect individuals with all types of structural anomalies against

elimination from the natural environment, particularly considering that information concerning this problem is only fragmentary (Mikulska & Jacuński, 1977; Jacuński & Napiórkowska, 2000). Epimorphosis in such cases is the subject of the present study.

Material and methods

Studies of epimorphosis of polymelic walking appendages and of parts of so-called complex anomalies of appendages were carried out on *Tegenaria atrica*. The particular developmental stages of this spider species have been defined according to the nomenclature adopted by Vachon (1957).

The experiments consisted of two stages. Before the first stage, whose objective was to obtain individuals with polymely and with complex anomalies of appendages, sexually mature individuals were collected from the natural environment. The spiders successively obtained in the years 1996–2002 were kept in the laboratory under conditions optimal for the species, i.e. at a temperature of 21–23°C and relative humidity of *c*. 70%. They were fed larvae of *Tenebrio molitor* (L.) and *Acheta domesticus* (L.) twice a week. After a short adaptation period each mature female was kept with different males, several hours with each, to avoid losses resulting from insufficient fertilisation. When oviposition started, each clutch was exposed alternately to temperatures of 14 and 32°C. The temperature was changed every 12 hours for 10 days, i.e. from the moment of oviposition until the first metameres of the prosoma appeared on the germ band. Then the developing embryos were kept at 23°C until the egg chorion and the prelarval membrane were cast off.

For the second stage of the experiments, individuals with polymely and with complex deformities of appendages were selected from the teratological material. Two days after they shed the prelarval membrane, the selected monsters had thin steelon thread ligatures put on their polymelic walking legs and on walking legs forming part of a more structurally complex appendage anomaly. The ligature loop was placed at the mid-length of the femur. In the case of polymely the experiment was extended by putting a ligature at the place of autotomy between the coxa and the trochanter in other individuals. The amputation ligature caused breakage in the continuity of the integument as well as of the muscles and nerves running in the appendages. In this way the haemocoel was also sealed, which made it possible for the spider to throw off the dead part of the appendage during the next moult. The operated individuals were then placed separately in Petri dishes, where they were kept through all successive postembryonal stages until the completion of the observations.

Throughout the experiments the experimental individuals were regularly supplied with drinking water and food. At first the food consisted of newly laid spider eggs, then small *Tribolium* sp. and *Tenebrio molitor* (L.) larvae. At the same time, under similar experimental conditions, a control culture was maintained consisting of individuals with the same deformities of the prosomal

appendages as in the experimental individuals. All the experimental individuals were photographed in each stage of the experiment. Moreover, histological sections were made of the experimental individuals, in which the structure and architectonics of internal organs were analysed, in particular the overall structure, arrangement and condition of the nervous system. The material was fixed in Bouin's solution made up according to Zawistowski's (1986) recipe. In order to facilitate penetration of the fixative the redundant appendages and the opisthosoma were cut off, then the preparations with the fixative were put into a vacuum chamber for 2 h. They were put into the chamber again while they were saturated with a mixture of xylene and paraffin with a melting temperature of 52°C. The paraffin cubes with preparations were sliced 6 μm thick and after appropriate histological procedures stained with Delafield haematoxylin and eosin and mounted in euparal.

Results

In eight years of studies of the epimorphic potentiality of accessory walking appendages and of walking legs forming part of a complex deformity in *T. atrica*, *c*. 15,000 embryos were exposed to alternating temperatures.

The death rate among the embryos in these experiments was considerable (*c*. 20%). Among the larvae which emerged from the egg membranes, 1348 individuals were found with nearly all the monstrosities found so far in *T. atrica*. Most deformities concerned the prosoma and the structure and number of the feeding and walking appendages (*c*. 93%). Polymelic monsters accounted for only 3.75%, while individuals with more complex leg deformities occurred even more sporadically (1.44%).

Amputation of accessory walking appendages

Disturbances in the prosoma associated with an increased number of appendages occurred in 47 larvae, 32 of which had accessory walking appendages. Accessory feeding appendages appeared in only 15 individuals.

A total of 28 larvae were selected for studies of the epimorphosis of polymelic walking legs. The remaining four cases of polymely were used as controls. The polymely in all these individuals was single polymely, which consisted of the development of one accessory appendage. The accessory legs of the larvae were similar in their length and seven-joint structure to normal walking legs. In most cases, however, their coxae were displaced slightly toward the dorsal or ventral side (Fig. 1A); less frequently they lay in a line with the remaining walking legs of the prosoma. In the latter case the choice of the correct appendage for amputation was made after studying the arrangement of mid-gut caeca. As is known (Jacuński, 1971, 1984), one such caecum reaches the base of each normal walking appendage. Polymely, on the other hand, is a deformity in which the

volume of the suboesophageal part of the nervous system is enlarged by accessory neuromeres (Fig. 1B), but no caecum extends toward the accessory appendage (Fig. 2A: 5). So far only one case has been described of a larva in which an increase in the number of appendages was associated with a corresponding increase in the number of mid-gut caeca (Jacuński *et al.*, 2002).

Twenty polymelic larvae had ligatures put at the mid-length of the femur. After amputation at the nymph I stage (Vachon stage IV), all the experimental individuals retained only a fragment of the appendage, with a scar at the place of amputation. The central part of the usually flat or slightly bulging surface of the regeneration scar was generally darker than the more peripheral part, which might be evidence of more intense cuticularisation. However, no normal regeneration of the amputated parts was observed, nor did the missing joints appear after later moults. Consequently, throughout the experiment, along with four pairs of normally developed, fully functional walking appendages there was a short, immobile stump of the accessory appendage. Before the completion of the experiment, i.e. until the nymph V stage (after five post-larval moults), no changes were observed in the morphological structure of these parts of the polymelic appendages.
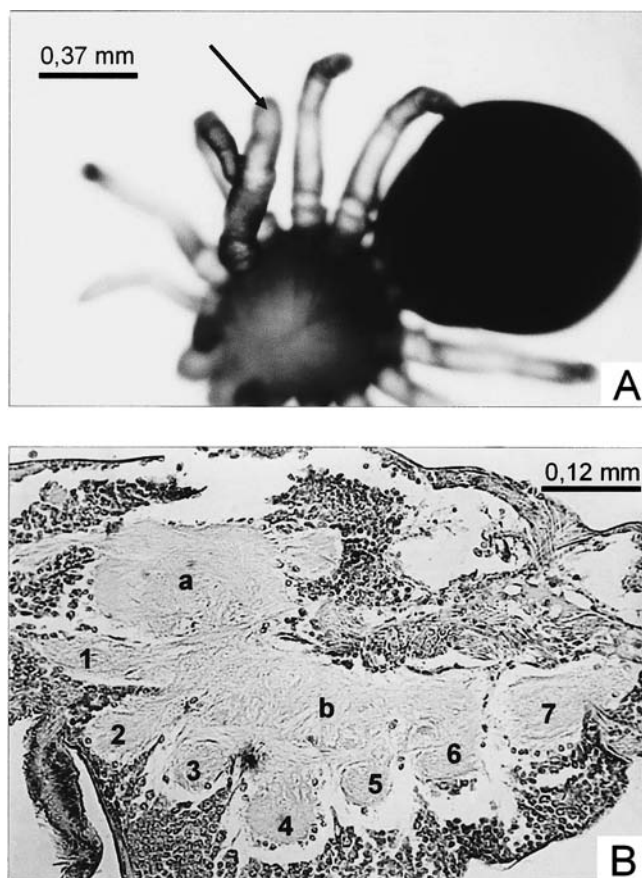

Fig. 1: **A** Larva of *Tegenaria atrica* with accessory walking appendage displaced to ventral side of prosoma (ventral view); **B** Longitudinal section through prosoma and central part of nervous system of *Tegenaria atrica* with accessory walking leg. Abbreviations: a=brain, b=suboesophageal part of nervous system, 1=ganglion of chelicera, 2=ganglion of pedipalp, 3–7=ganglia of walking legs.
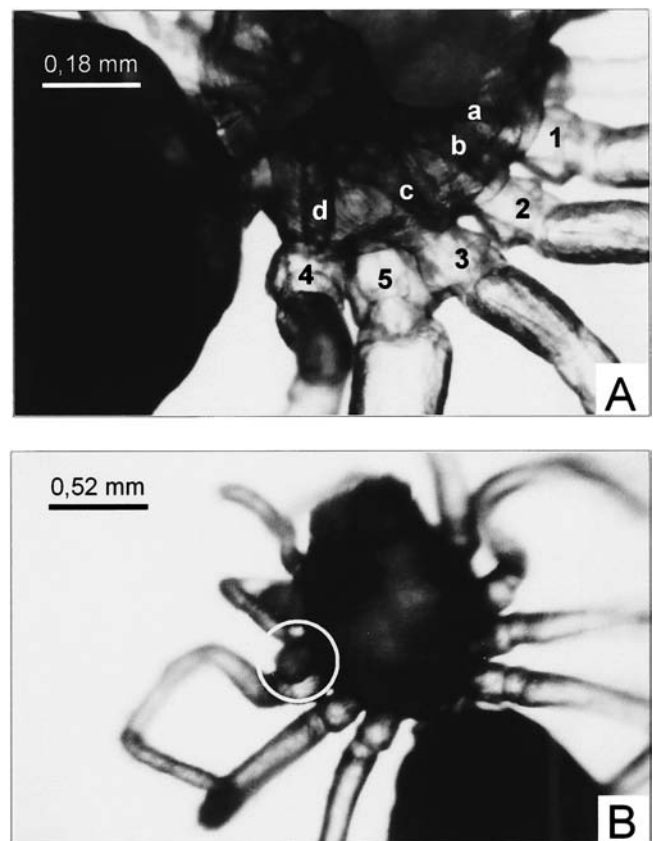

Fig. 2: **A** Polymelic larva of *Tegenaria atrica* (dorsal view); **B** Nymph II of *Tegenaria atrica* with four pairs of walking legs and coxal joint of accessory leg (ventral view). Abbreviations: 1–4=normal walking legs, 5=accessory walking leg, a–d=caeca of mid-gut.

In view of the fact that amputation of the accessory appendages at mid-length of the femora did not lead to epimorphic regeneration, the experiment was extended by amputating the appendages at the place of autotomy between the coxa and trochanter. This amputation was carried out in eight other polymelic individuals at the larval stage. The effect was the same as in the preceding variant of the experiment, i.e. no regeneration took place (Fig. 2B).

Observations of individuals in the control sample showed that in further ontogeny the polymelic appendages did not develop properly. Their growth rate was much slower than that of the remaining walking appendages. At the nymph I stage the accessory appendages were *c.* 82% of the length of the remaining, normally developing walking appendages, while at the nymph II stage (after two post-larval moults) that proportion was only 75%. While normal appendages were equipped with large pectinate claws at the nymph I stage, they did not appear on the accessory appendages until the nymph II stage. The accessory appendages also differed from the normal ones in that they did not participate in the spiders' locomotion. In one individual of the control group the accessory appendage was autotomised at the first nymphal moult. Also in that case no regeneration was observed before the completion of the experiment.

The death rate in this series of experiments was *c.* 21% (six individuals). The accessory appendage of one of the larvae was injured in the course of the ligature

procedure, and this caused excessive loss of haemo-lymph. For the remaining five spiders the critical time was the first post-larval moult.

Amputation of fragments of appendages in complex anomalies

Complex anomalies consisted of the simultaneous occurrence in one individual of two or more deformities, such as schistomely (bifurcation of appendage), hetero-symely (accretion of legs lying on one side of the body), polymely (increase in number of legs), oligomely (reduction of number of legs) and symely (accretion of legs lying on opposite sides of the prosoma). In the whole experimental period 18 larvae with complex appendage anomalies were obtained. The appendages involved in the anomalies were either both the walking and feeding appendages or only the walking legs while the feeding appendages remained unchanged in number and in morphological structure. Only five individuals (*c.* 28%) were selected for studies of epimorphic potentiality. In all these larvae the coxal joints of the pedipalpi were accreted with the coxae of the first walking legs, whose more distal parts were bifurcate (Fig. 3A). The remaining cases of complex anomalies were excluded from the experiment because of their non-recurrence.

The ligatures were applied on the second day after hatching. Amputation was carried out at the mid-length of the femora of those walking legs which showed single schistomely in their distal parts (Fig. 3B).

At the nymph I stage well-scabbed scars were observed on the amputation surface. As in individuals with an accessory walking leg, the scars were usually flat; in only one case was there a central protuberance on the bulging surface. At the nymph II stage (after two post-larval moults) in cases of complex anomalies no structural changes were observed at the place where after amputation of a leg fragment the first regenerate usually appears (Fig. 3C). Also no regeneration was observed at the nymph III stage (Fig. 3D) or in the two following developmental stages. Before the completion of the experiment the only change observed was an elongation of the stumps, proportional to that of the other walking appendages. All spiders lacking a fragment of leg moved adroitly about the web.

Out of the five operated individuals one died as a result of haemolymph loss while throwing off the cuticle in the first post-larval moult. The remaining four reached the nymph V stage.

Discussion

The experimental study of the epimorphic potential of polymelic walking appendages and of those forming parts of so-called complex deformities of appendages in *T. atrica* consisted of two parts. The object of the first stage was to induce the above deformities by exposing developing embryos to alternating temperatures (14 and 32°C). That was followed by the second stage of studies directly concerning the regeneration process.
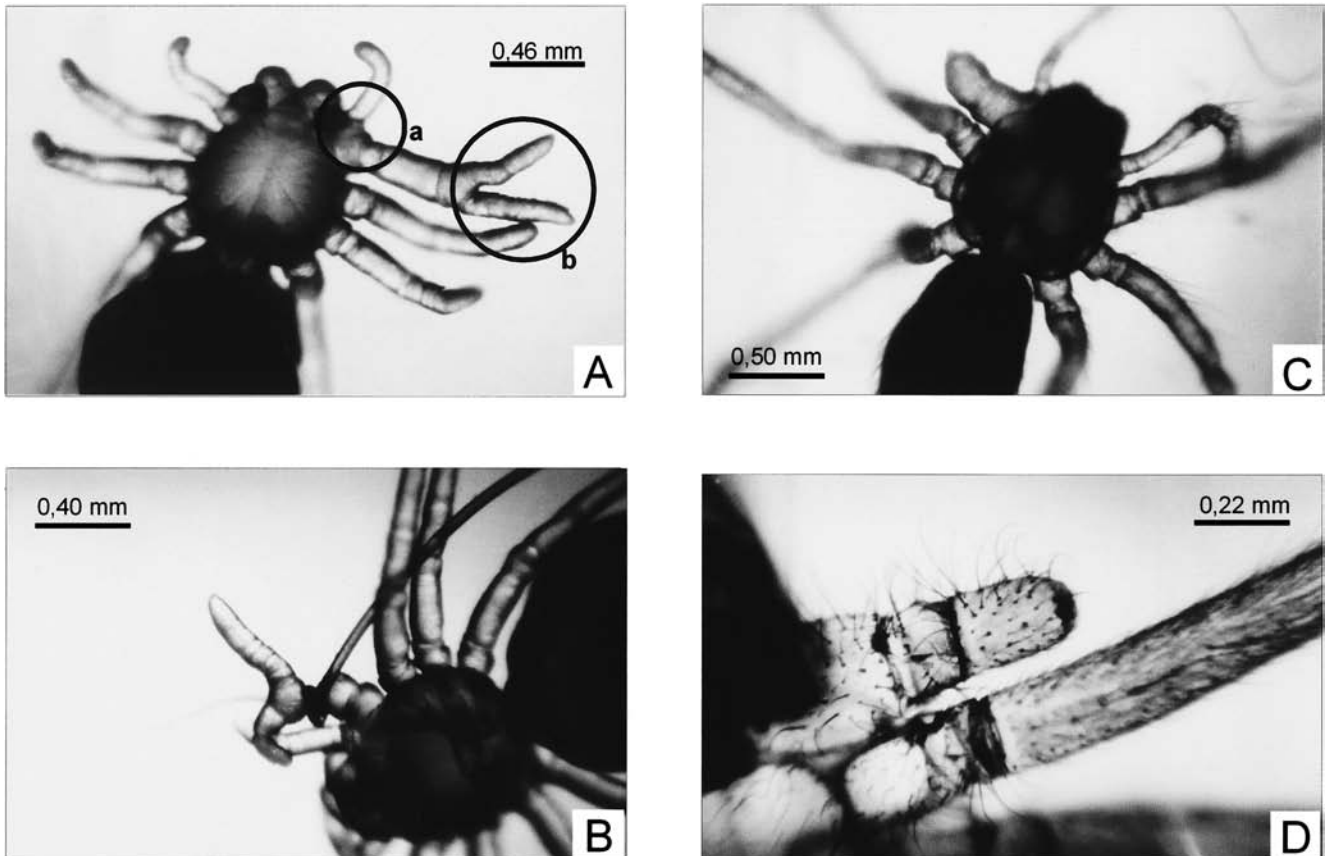

Fig. 3: **A** Shallow heterosymely of pedipalp and schistomelic walking leg in *Tegenaria atrica* larva, ventral view (a=accreted coxae, b=bifurcate walking leg); **B** Larva of *Tegenaria atrica* with complex anomaly of appendages, with ligature on schistomelic walking leg (dorsal view); **C** Nymph II without regenerate of part of the appendage complex (dorsal view); **D** Individual without regenerate of part of the appendage complex at nymph III stage (dorsal view).

Before the observation of changes involved in the regeneration process, amputation was carried out by means of steelon thread ligatures. Tightening the ligature broke the continuity of the tissues, and the part of the leg distal to the ligature was subject to necrosis induced by cutting off the haemolymph supply. During this time the wound was cicatrising and being coated with a darker, and therefore more intensely cuticularised, integument.

In the experiment with polymelic individuals whose accessory leg was amputated at the mid-length of the femur, no normal jointed external regenerates were found before the completion of observations, i.e. until the nymph V stage. Since no regenerates developed at the place of amputation, eight other larvae obtained from teratological material were operated on at the point of autotomy between the coxa and trochanter. The aim of that change in research method was to find out whether amputation at that place, specific for the epimorphosis of each appendage (Bauer, 1972) would initiate the process of regeneration of the amputated joints. The result, however, was also negative. In this case, too, the wounds were only scabbed. This process can be regarded as the initial stage of the regeneration process in arthropods, preceding the development of the lost part of the appendage. In the above experiments it therefore represented the first and last noticeable effect of amputation, protecting the spider against haemolymph loss. Evidence of the importance of this process may be provided by the fact that the cuticle layer forming over the place of amputation was thicker than normal. Since no external regenerates were found, it can be inferred that accessory appendages, which do not occur in normal ontogeny, have limited regeneration potentialities. Their development potential in further postembryogeny is also limited, evidence of which was found in the observations on the control individuals. The obvious reason for this is that the development of one or more accessory appendages considerably disturbs the overall structure of the prosoma, thus handicapping the basic vital activities, such as locomotion and obtaining food. To some extent it would also reduce the potential for reaching sexual maturity and procreation. The best solution of the problem would therefore be the elimination (autotomy) of the superfluous legs with no potential for their being reconstructed. That developmental strategy would seem to be an important evolutionary achievement for arthropods.

The above experiments are in line with the case described earlier by Jacuński et al. (2002) of a spider in which autotomy of an accessory walking appendage occurred in the course of nymphal ecdysis. In that particular case, likewise, no changes were observed which provided evidence of epimorphic processes leading to the reconstruction of the cast-off fragment of the polymelic appendage. It can therefore be inferred that the accessory leg, despite its well-developed innervation, shown in detailed histological studies (Fig. 1B), is not subject to the same developmental laws as a normally developed appendage (Jacuński et al., 1994).

The above statements, however, do not apply to the appendages which in disturbed embryogeny can develop on the petiolus. It is known that during embryonic development appendage buds can develop at that place on the opisthosoma, but in normal embryogeny they soon disappear (Jacuński, 1984). However, under the influence of teratogenic factors these buds continue developing to form jointed appendages. After their amputation a normal regeneration process has been observed, sometimes recurring several times and leading to full reconstruction (Jacuński & Templin, 1991). It can be assumed that the capability for epimorphosis of such legs is possible since, unlike the accessory appendages of the prosoma, they have a reference to ontogeny. Similar epimorphic potential is found in the spinnerets, which develop from appendage buds on the opisothosomal metameres (Bonnet, 1930; Ivanov, 1965).

Also, no epimorphic regenerates were observed after amputation of bifurcate appendage fragments in complex anomalies. Until the nymph V stage only well scabbed post-amputation scars could be seen at the place of amputation, which may be evidence of a considerable narrowing of the spectrum of the effect of morphogenetic mechanisms. A different result was obtained after autotomy of an appendage complex in T. atrica by Jacuński & Napiórkowska (2000). After it was torn off at the point of autotomy in the course of the third nymphal moult, in the following developmental stage there appeared a short regenerate of the larval type, which showed no trace of earlier deformity. Moreover, in the nymph VI stage it reached the same size as the well-developed corresponding appendage on the opposite side of the prosoma. Its further autotomy, which took place several days after the moult from stage VI to stage VII, induced renewed activation of epimorphic mechanisms, the result of which was the development of another regenerate of the larval type in stage VII. That regenerate was almost immediately subject to autotomy, and the same happened after the four following moults. These newly reappearing short regenerates were torn off. It can therefore be assumed that in some cases appendages affected by complex anomalies can undergo epimorphosis, although the regenerates do not always reach their final shape. Sometimes the effect of the ballast they constitute for the animal becomes too great and they are removed. The question remains, why in only one particular case did an external regenerate develop? It may have some connection with the particular type of complex anomaly. In the study quoted above the anomaly concerned only walking appendages (simultaneously polymely, heterosymely and schistomely), while in the experiment described in the present paper the appendages concerned were different both in structure and in function, namely the pedipalp and the first walking appendage. That may have been the barrier not allowing a full regeneration process.

The above experiment has demonstrated that polymely and other more complicated deformities of appendages cause a reduction or complete loss of potential for their regeneration. There is, however, no doubt that leg regeneration is in every respect a very important,

commonly occurring morphogenetic process, extremely strongly set in the strategy of the postembryonal development of spiders. That process, however, refers fully only to the prosoma appendages, whose presence is secured by a long-lasting evolution process.

References

BAUER, K. H. 1972: Funktionsmechanismus der Autotomie bei Spinnen (Araneae) und seine morphologischen Voraussetzungen. *Z. Morph. Tiere* **72**: 173–202.

BONNET, P. 1930: La mue, l'autotomie et la régénération chez les araignées, avec une étude des Dolomédes d'Europe. *Bull. Soc. Hist. nat. Toulouse* **59**: 237–700.

FRIEDRICH, P. 1906: Regeneration der Beine und Autotomie bei Spinnen. *Arch. EntwMech. Org.* **20**: 469–506.

GABRITSCHEVSKY, E. 1927: Experiments on color changes and regeneration in the crab-spider, *Misumena vatia*. *J. exp. Zool.* **47**: 251–266.

GABRITSCHEVSKY, E. 1930: Les réductions régulatrices et les compensations hypertrophiques pedant l'ontogenése et la régénération de l'araignée *Thomisus onustus*. *Bull. biol. Fr. Belg.* **64**: 155–190.

HOLM, A. 1940: Studien über die Entwicklung und Entwicklungsbiologie der Spinnen. *Zool. Bidr. Upps.* **19**: 1–214.

IVANOV, A. V. 1965: *Pająki, ich budowa, życie i znaczenia dla człowieka* [*Spiders, their morphology, biology and economic importance*]. 1–304. Univ. Leningrad. [In Russian.]

JACUŃSKI, L. 1969: The effect of temperature treatment at various stages of embryonic development on formation of monstrosities in the spider *Tegenaria atrica*. C. L. Koch. *Bull. Acad. pol. Sci. Ser. Sci. biol.* **17**(9): 555–556.

JACUŃSKI, L. 1971: Temperature induced developmental monstrosities in *Tegenaria atrica* C. L. Koch (Araneae, Agelenidae). *Zoologica Pol.* **21**: 285–316.

JACUŃSKI, L. 1984: *Studia nad teratogenezą eksperymentalną u pająka* Tegenaria atrica *C. L. Koch*. Toruń, UMK.

JACUŃSKI, L. 2002: Anomalies of the abdomen in *Tegenaria atrica* C. L. Koch (Araneae, Agelenidae). *Bull. Pol. Acad. Sci. Biol.* **50**(3): 183–188.

JACUŃSKI, L. & NAPIÓRKOWSKA, T. 2000: Epimorphic regeneration of appendage complex in *Tegenaria atrica* C. L. Koch (Agelenidae). *Bull. Pol. Acad. Sci. Biol.* **48**(3): 269–271.

JACUŃSKI, L., NAPIÓRKOWSKA, T., TEMPLIN, J. & TESZNAR, L. 2002: Interesting cases of polymely in *Tegenaria atrica* C. L. Koch (Agelenidae). *Bull. Pol. Acad. Sci. Biol.* **50**(2): 149–151.

JACUŃSKI, L., NAPIÓRKOWSKA, T., TEMPLIN, J. & TESZNAR, L. 2004: Anomalies in the cephalic part of prosoma in *Tegenaria atrica* C. L. Koch. *Zoologica Pol.* **49**(1–4): 97–110.

JACUŃSKI, L. & TEMPLIN, J. 1991: Odnóże na petiolus u pająka *Tegenaria atrica* C. L. Koch w rozwoju postembrionalnym. [Leg on the petiolus of a spider *Tegenaria atrica* C. L. Koch in the postembryonic development]. *Przegl. zool.* **35**(1–2): 135–138. [In Polish, English summary.]

JACUŃSKI, L. & TEMPLIN, J. 2003: Morphology of prosoma in bicephalous monsters of *Tegenaria atrica* C. L. Koch. *J. therm. Biol.* **28**: 393–396.

JACUŃSKI, L., WIŚNIEWSKI, H. & TEMPLIN, J. 1994: The epimorphosis of a walking leg in the early stages of postembryogeny in *Tegenaria atrica* C. L. Koch (Arachnida, Agelenidae). *Zesz. nauk. WSP, Bydgoszcz, Stud. Przyr.* **11**: 135–163.

JUBERTHIE, C. 1962: Etude des symélies provoquées par la température chez un Opilion (Arachnide). *C. r. hebd. Séanc. Acad. Sci., Paris* **254**: 2674–2676.

JUBERTHIE, C. 1963a: Production expérimentale de l'hétérosymélie chez un Opilion. *C. r. hebd. Séanc. Acad. Sci., Paris* **256**: 3363–3365.

JUBERTHIE, C. 1963b: Monstruosités observées chez les Opilions. *Bull. Mus. natn. Hist. nat., Paris* (2) **35**(2): 167–171.

JUBERTHIE, C. 1968: Tératologie expérimentale chez un Opilion (Arachnide). *J. Embryol. exp. Morph.* **19**(1): 49–82.

JURA, C. & KRZANOWSKA, H. 1998: *Encyklopedia biologiczna*. Kraków.

LOCKET, G. H. 1936: Regeneration in Arachnida. *Nature, Lond.* **138**: 885–886.

MIKULSKA, I. 1973: Experimentally induced developmental monstrosities in the water spider *Argyroneta aquatica* (Clerck). *Zoologica Pol.* **18**: 97–106.

MIKULSKA, I. & JACUŃSKI, L. 1970: A two-headed monster of the spider *Tegenaria atrica* C. L. Koch. *Acta arachn. Tokyo* **23**(1): 17–19.

MIKULSKA, I. & JACUŃSKI, L. 1971: Dichotomy in embryos of the spider *Tegenaria atrica* C. L. Koch induced by supraoptimal temperature treatment in early embryogeny. *Zoologica Pol.* **21**: 281–284.

MIKULSKA, I. & JACUŃSKI, L. 1977: The regulating processes after the amputation of teratologically changed appendages in *Tegenaria atrica* C. L. Koch (Araneae, Agelenidae). *Zoologica Pol.* **26**: 45–50.

MIKULSKA, I., JACUŃSKI, L. & WEYCHERT, K. 1975: The regeneration of appendages in *Tegenaria atrica* C. L. Koch (Agelenidae, Araneae). *Zoologica Pol.* **25**: 99–110.

OPPENHEIM, S. 1908: Regeneration und Autotomie bei Spinnen. *Zool. Anz.* **33**: 56–60.

RANDALL, J. B. 1981: Regeneration and autotomy exhibited by the black widow spider, *Latrodectus variolus* Walckenaer. *Wilhelm Roux Arch. dev. Biol.* **190**: 230–232.

ROTH, V. D. & ROTH, B. M. 1984: A review of appendotomy in spiders and other arachnids. *Bull. Br. arachnol. Soc.* **6**: 137–146.

RUHLAND, M. 1976: *Die neuromuskulare Organisation normaler und regenerierter Laufbeine einer Vogelspinne* (Dugesiella hentzi *Ch., Orthognatha, Aviculariidae*). Dissertation, Universitat Konstanz, Germany.

SAVORY, T. H. 1936: Regeneration in Arachnida. *Nature, Lond.* **138**: 550.

VACHON, M. 1957: Contribution a l'étude du dévelopment postembryonnaire des araignées. Première note. Généralités et nomenclature des stades. *Bull. Soc. zool. Fr.* **82**: 337–354.

VACHON, M. 1967: Nouvelles remarques sur la régénération des pattes chez l'araignée *Coelotes terrestris* Wid. (Agelenidae). *Bull. Soc. zool. Fr.* **92**: 417–428.

VOLLRATH, F. 1990: Leg regeneration in web spiders and its implications for orb weaver phylogeny. *Bull. Br. arachnol. Soc.* **8**(6): 177–184.

WEISS, O. 1907: Regeneration und Autotomie bei der Wasserspinne (*Argyroneta aquatica* Cl.). *Arch. EntwMech. Org.* **23**: 643–645.

WOOD, F. D. 1926: Autotomy in Arachnida. *J. Morph.* **42**: 143–195.

ZAWISTOWSKI, S. 1986: *Technika histologiczna, histologia oraz podstawy histopatologii*. Wyd. 5., PZWL, Warszawa.