Disturbances in the structure of the prosoma in *Tegenaria atrica* induced by alternating temperatures (Araneae: Agelenidae)

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Summary

Some unique cases of bicephality in *Tegenaria atrica* C. L. Koch are described. This phenomenon is known to be the result of broadening and then sagittal bifurcation of the anterior germ band. In consequence there develop two head tagmae, equivalent if the germ band divides symmetrically or non-equivalent if the division is asymmetrical. The bifurcation of the germ band can be shallow, which produces two heads, or deep, which may lead to the development of so-called "Siamese twins". A less frequent cause of bicephality is bifurcation across the anterior end of the germ band in the horizontal plane. Then two head somites develop, the main head, usually with a normal structure, and a so-called accessory head, usually incomplete, situated one above the other. In the authors' opinion the study of the morphology of the anterior body in bicephalous individuals provides exceptional opportunities for studying the interrelations between the functioning of the particular parts of the brain and widely understood behaviour of spiders.

Introduction

The head in spiders is a body tagma, whose metameric structure is still the subject of discussion (Legendre, 1979; Averof, 1998; Damen *et al*., 1998; Telford & Thomas, 1998). One of the methods aimed at elucidating the morphology of that part of the body is disturbing its morphogeny, leading to developmental anomalies. However, producing disturbances in the head region experimentally is not easy, as the anterior prosoma shows exceptional resistance to the teratogenic effect of temperature. In the region of the head tagma some isolated deformities have been observed, such as oligomely (reduced number of appendages), polymely (increased number of appendages), schistomely (bifurcation of appendages), and accretion of appendages (symely and heterosymely) (Jacuński, 1971, 1984; Jacuński *et al*., 2004). Most spiders with such simple anomalies have a chance of prolonged survival, as they easily pass through successive nymphal ecdyses. That, however, does not apply to bicephality of the "duplicitas anterior" type (Holm, 1940; Sekiguchi, 1957). Experimentally obtained bicephalous spiders occur only sporadically, and their survival rate is extremely variable. Therefore each new morphological form of a bicephalous individual is worth publishing. A thorough study of these anomalies serves as an introduction to further research on the physiology of the spider brain and on the regeneration process in that part of the body. It has been found that bicephalous spiders have two independent brains. In such cases amputation of one of the heads with the central part of the nervous system or part of it provides unique opportunities for studying the reciprocal relations between the functioning of the particular parts of the brain and the spiders' broadly understood behaviour.

The present paper describes several individuals of *Tegenaria atrica* C. L. Koch with a particularly complex structure of the anterior prosoma. These were individual, unique monstrosities, which occurred extremely rarely in our teratological studies and are therefore difficult to interpret morphogenetically.

Material and methods

Our experimental studies were done on embryos of *Tegenaria atrica*, which belongs to the family Agelenidae and is one of the largest synanthropic spiders in Poland. Sexually mature individuals were collected in summer (July–September) in the area near Toruń, then kept in a laboratory colony until the end of the breeding season, under conditions optimal for the species. The laboratory was shaded, with an ambient temperature of 21–23 °C and relative humidity of *c*. 70%. Sexually mature spiders were placed separately in glass jars of 250 cm^3 capacity and fed every other day on larvae of *Tenebrio molitor* (L.) and nymphs of *Acheta domesticus* (L.). After a period of adaptation to the laboratory conditions lasting for about a fortnight each female was kept with several males in turn in order to maximise insemination. After the start of oviposition the eggs of each batch were divided into two parts, one of which served as control (*c*. 7,000 embryos) while the other (*c*. 7,000 embryos) was exposed to thermal shock at alternating temperatures of 14 and 32 °C at a relative humidity of *c*. 70%. The temperature was changed every six hours. Incubation at alternating temperatures was run from the moment of oviposition until the appearance on the germ band of the first metameres, which later form the prosoma. Then the embryos were kept at a temperature of 23 °C until the larvae left the egg capsules. The whole process of embryogeny was observed in the experimental and control groups. The developing eggs were immersed in paraffin oil, which effectively illuminates the egg membranes and makes it possible to observe the developing embryos.

Results

From among the larvae those showing anomalies of the anterior prosoma not so far described in the teratological literature were selected for further observation.

The first two spiders were bicephalous individuals with the whole prosoma considerably extended. In each of them there was a properly structured head, which was called the main head, and a head with modified morphological structure, which was called the side head. In spider 1 (Fig. 1) the main head seen from the ventral side was situated on the left side of the body and was slightly bent towards the dorsal side. Both lips of the mouth as well as the chelicerae (a, a) and the pedipalpi (b, b) were normally developed. The side head was situated on the right side of the anterior part of the prosoma. The basal joints of its chelicerae (a′) were longitudinally accreted

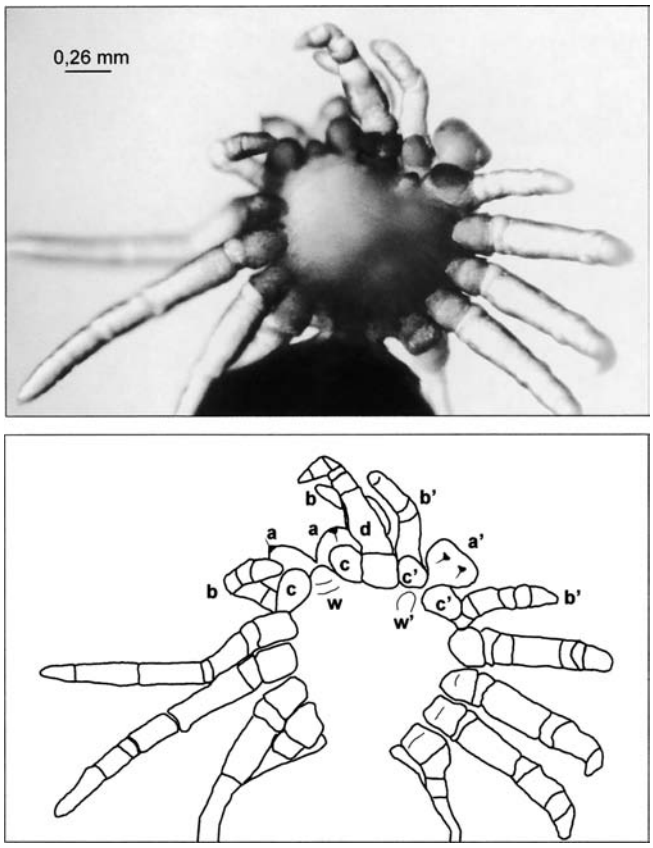

Fig. 1: Bicephalous larva of *Tegenaria atrica* with main head and side head (ventral views). Main head: a=chelicera, b=pedipalpus, c=gnathocoxa, w=lower lip. Side head: a′=symelic chelicerae, b′=pedipalpus, c′=gnathocoxa, w′=lower lip, d=accessory walking leg.

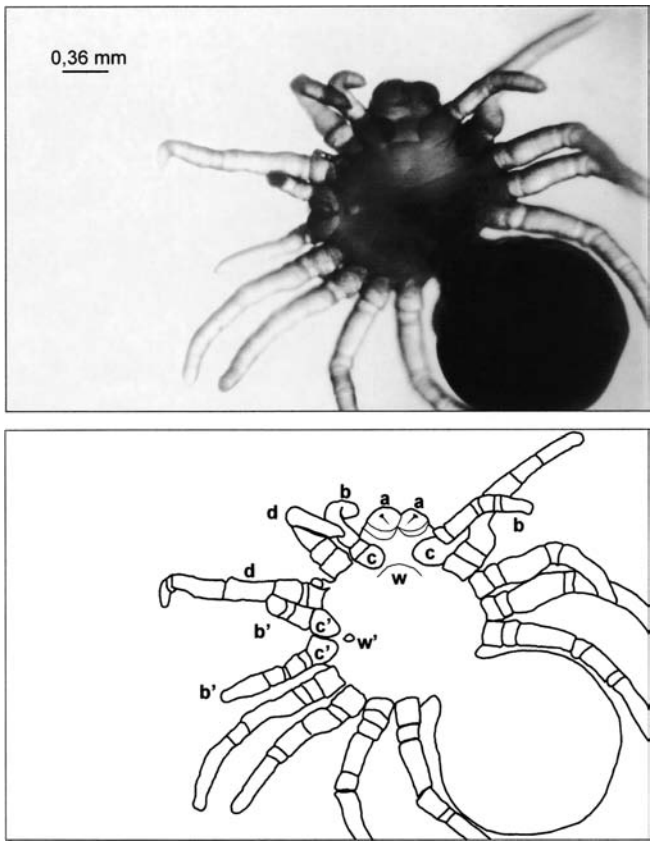

Fig. 2: Bicephalous larva of *Tegenaria atrica* with main head and side head (ventral views). Main head: a=chelicera, b=pedipalpus, c=gnathocoxa, w=lower lip. Side head: b′=pedipalpus, c′=gnathocoxa, w′=lower lip, d=accessory walking legs.

(symely), while the two claws at the tip were regularly formed. The pedipalp (b′, b′) of that head were normally formed and situated. In the mouth system the upper lip was missing and the lower lip had developed in the form of an elongate capsule (w′). Between the two heads there was an accessory walking appendage (d) with a swollen ball-shaped coxa. The spider lived only three days. Bicephalous spider 2 (Fig. 2) reached nymph II stage before it died. The main head had a regular size and structure. It was situated on the right side (as viewed ventrally) of the main body axis. The side head, situated on the left side of the anterior prosoma (viewed ventrally) was incomplete. It lacked the chelicerae and the upper lip. In place of the lower lip there was a small promontory — probably the transformed lower lip (w′). On the dorsal side of that head there was one eye and next to it a single massive seta. Only the pedipalpi (b′) of the side head were properly formed. Between the two heads there were two accessory normally developed walking legs (d) and a small stump-like appendix, which atrophied before the spider reached nymph II stage.

In spider 3 (Fig. 3) disturbed embryogeny resulted in the development of two complete prosomal tagmae coadunate at an angle of 180°, linked to the common opisthosoma by a broad petiolus. On the dorsal side each of the coadunate tagmae had its own carapace. In the anterior part of each prosoma there was an eye complex on the dorsal side and a mouth with lips on the ventral side. The number of both feeding and walking appendages was also complete. The arrangement of the walking legs was normal except for legs IV, which were situated on the external edges of the dichotomous prosoma. Because of shortage of space, the coxae of those appendages had been shifted towards the dorsal side. In this individual the mid-gut caeca, whose number and arrangement corresponded with those of the walking legs, developed normally. On the ventral side a broad groove ran paracentrally between the dichotomous prosomae and the broad petiolus.

The prosoma of spider 4 (Fig. 4) was distinctly broader than in control individuals and slightly convoluted anteriorly. It had two heads in the anterior part of

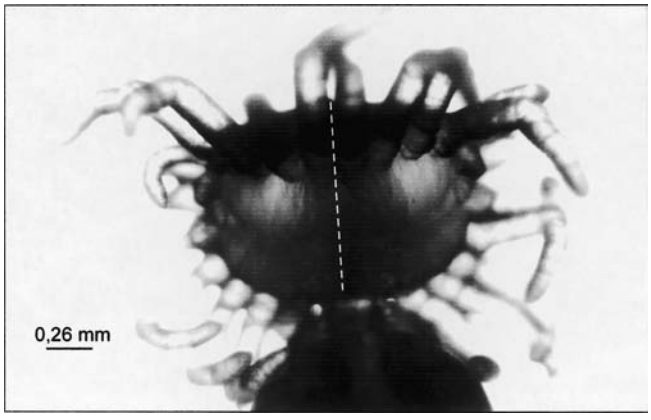

Fig. 3: Larva of *Tegenaria atrica* with dichotomous prosomal tagma (ventral view).

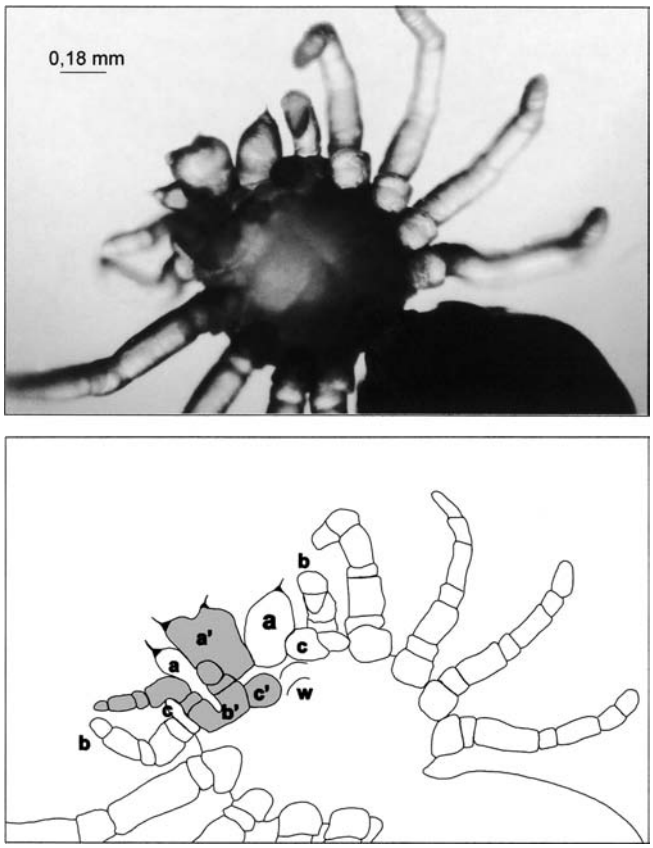

Fig. 4: Bicephalous larva of *Tegenaria atrica* with main and accessory heads arranged one above the other (ventral views). Main head: a=chelicera, b=pedipalpus, c=gnathocoxa, w=lower lip. Accessory head (dark grey): a′=symelic chelicerae, b′=schistomelic pedipalpus, c′=gnathocoxa.

the body: the main one which was only slightly modified in structure, and an accessory one with a reduced number of appendages. The main head had a pair of normal, though slightly sideways shifted, chelicerae (a, a). The right (as viewed ventrally) pedipalpus (b) was normally situated, while the left one (b) was shifted away from the lower lip (w) as a result of the accessory head getting wedged in. The cheliceral and pedipalpal metameres forming the accessory head section were situated nearer the ventral side. The chelicerae (a′) of the accessory head were accreted (symely), and at their tips there were claws turned in opposite directions. The accessory head had only one pedipalpus (b′), which was bifurcate (schistomely) up to the mid-coxa. At its base there was a single gnathocoxa (c′).

Spider 5 (Figs. 5–6) was a quite exceptional bicephalous individual. As in the former cases, its prosoma was much broader than usual. There were also two heads on the front edge of the prosoma. The main head was completely formed. It included a pair of normally developed chelicerae (a, a) arranged obliquely in relation to the frontal plane. There was also a pair of normally jointed pedipalpi (b, b) equipped with normally sized gnathocoxae (c); only the right (as viewed ventrally), pedipalpus (b) was considerably shifted towards the right side of the body. The lips of the mouth were normally sized. On its dorsal side (Fig. 6) there was a complete set of eyes arranged in the way characteristic of the species. The accessory head was situated beneath the main head and had a pair of normally developed chelicerae (a′, a′) arranged obliquely to the frontal plane. The bicephalous spider had an accessory pair of symelic pedipalpi (b″) with two normally sized gnathocoxae (c″). The lower lip (w′) on the accessory head was slightly shifted towards the left (as viewed ventrally) side of the body. On its dorsal side (Fig. 6) there was an incomplete eye complex with three eyes lying close together.

Discussion

Disturbances in the structure of the anterior body, consisting of its bifurcation, can be caused by various teratogenic factors. Developmental anomalies of this type have been induced using, among other things, egg centrifuging. Dichotomous embryos of *Araneus nauticus* (L. Koch) were thus obtained by Sekiguchi (1957). According to that author, dichotomy of part of the body occurs most frequently in embryos subjected to that treatment from the stage of blastoderm formation until that of the second blastoderm contraction, and can affect both the front and rear of the body. Sekiguchi worked out a classification of the different types of dichotomous embryos. Similar studies were conducted by Ehn (1964) on embryos of *Agelena labyrinthica* (Clerck). By treating the embryos with centrifugal force he obtained several types of dichotomous individuals.

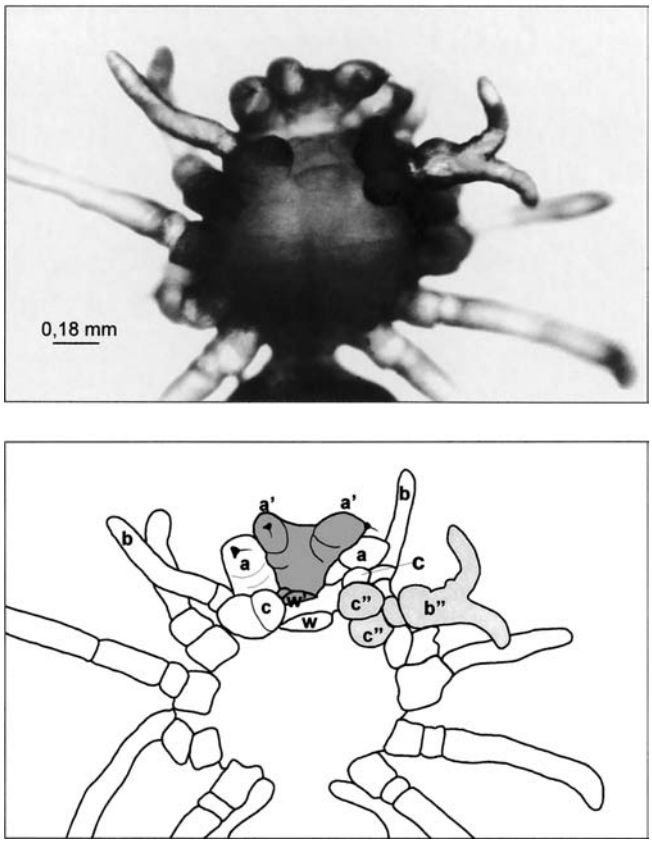

Fig. 5: Larva of *Tegenaria atrica* with main head and accessory head arranged one above the other (ventral views). Main head: a=chelicera, b=pedipalpus, c=gnathocoxa, w=lower lip. Accessory head (dark grey): a′=chelicerae, w′=lower lip, b″=additional symelic pedipalpi (light grey), c″=gnathocoxae of additional pedipalpi.

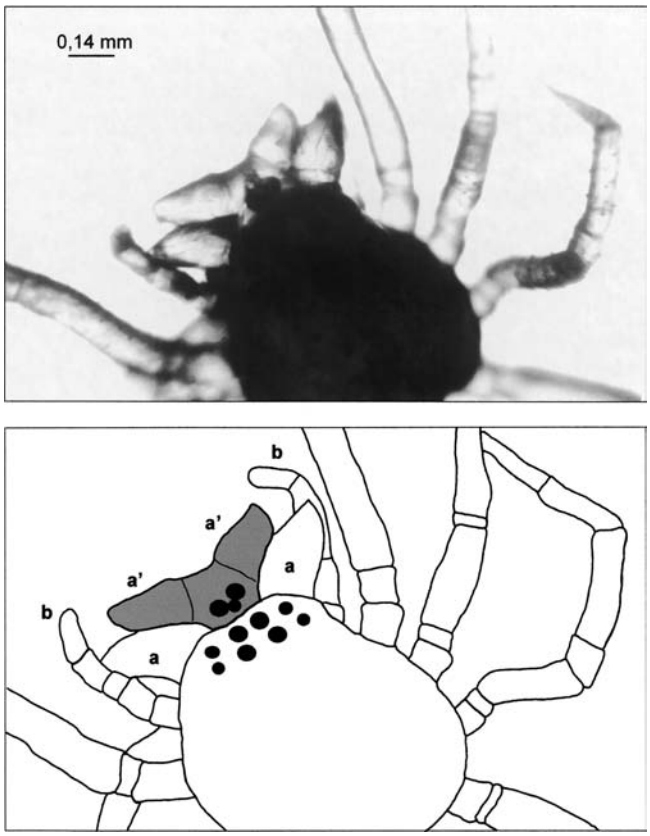

Fig. 6: Larva of *Tegenaria atrica* with main head and accessory head arranged one above the other (dorsal views). Main head: a=chelicera, b=pedipalpus. Accessory head (dark grey): a′=chelicerae.

He also found that the stage most susceptible to centrifugation was the gastrulation stage. According to Ehn, centrifugation causes the cell material, which forms a cumulus, to divide into two parts, each of which behaves as a separate posterior-ventral organisation centre of the embryo's axial system. Seitz (1966), on the other hand, treated young embryos of *Cupiennius salei* (Keyserling) with X-rays. The initial effect of that irradiation, depending on the size of the dose, was impairment at the cell level, and then in the embryo's layer system, i.e. in the yolk-plasma systems. The effects of using even higher doses were irreversible changes in the yolk, leading to the destruction of the cohesion of the main layers of the young embryo, necessary for its normal development. Using local, i.e. barrier, irradiation of embryos in the blastoderm stage, he obtained embryos with a bifurcate anterior body, i.e. bicephalous embryos.

A disadvantage of all the above-mentioned experiments was the fact that the relevant deformities could be observed only in the course of embryogeny. The impaired embryos obtained by such drastic methods died before leaving their egg capsules, which made it impossible to continue the observations of anomalies in postembryogeny. On the other hand, using temperature as the factor inducing anomalies makes it possible to obtain dichotomous individuals able to continue postembryonal life. This method was first used in spiders by Mikulska & Jacuński (1970, 1971) and by Jacuński (1971, 1984, 1992).

Among individuals with deep anomalies in the anterior prosoma induced by temperature high mortality was noted in postembryogeny. The direct cause was probably not the teratogenic factor, i.e. alternating temperatures, but a combination of different types of monstrosities, which produced a lethal effect in the initial phase of postembryogeny, often making impossible an anatomical verification of the anomalies.

Earlier studies of disturbed embryogeny have demonstrated that the main cause of bicephality of the "duplicitas anterior" type is bifurcation in the sagittal plane or broadening of the germ band, which can be symmetrical or asymmetrical. In the case of symmetrical bifurcation two equivalent heads with a complete structure are developed. In the case of an uneven division of the cell material two different heads are developed, the main head and a side one. The main head usually has a normal structure. The side heads, on the other hand, are generally incomplete: they show a reduced number of eyes, a lack of or deformities of the feeding appendages and of the lips surrounding the mouth (Jacuński & Templin, 1992, 2003; Jacuński, 2002). Sometimes, when the bifurcation of the germ band is deeper, one or more walking appendages develop between the two heads. The effect of the extremely rare cases of dichotomy of the whole germ band is not only bicephality but dichotomy of the whole body and the development of so-called "Siamese twins" (Mikulska & Jacuński, 1971; Jacuński, 2002).

In two of the bicephalic individuals (Figs. 4–6) the heads were situated one above the other. In these cases the accessory head tagma was called the additional head. Such an arrangement of heads has not been observed before; it develops probably as a consequence of bifurcation of the anterior part of the germ band in the horizontal plane. That hypothesis requires further studies.

The individuals under study also showed other disturbances in the structure of the anterior prosoma besides bicephality. These probably resulted from disturbances in the particular stages of the formation of metameres in the developing prosoma. In the species studied they form in a strictly defined order. The first to form are the four thoracic metameres, then the pedipalpal metamere, and lastly the cheliceral metamere (Ivanov, 1965; Yoshikura, 1954, 1955). At each stage disturbances may occur, as demonstrated by Jacuński (2002).

A comprehensive interpretation of the anomalies developing in the anterior part of the spiders' body is extremely difficult, as spiders do not have a separate cephalic tagma. It is inseparably linked with the thoracic part. Besides, the final metamere composition of that tagma in Chelicerata is still being discussed and compared with that in other Arthropoda. Studies of the expression pattern of Hox genes are used for that purpose (Averof, 1998; Damen *et al.*, 1998; Telford & Thomas, 1998). The results of these studies have changed earlier views on the homology of the prosomal metamery in Chelicerata and other Arthropoda but do not elucidate all the causes contributing to the formation of the particular parts of the body in spiders.

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