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Bicephality, a seldom occurring developmental deformity in *Tegenaria atrica* caused by alternating temperatures

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ABSTRACT

The experiment was aimed at demonstrating the relationship between deformities of the front part of the prosoma accompanied by changes in the brain structure in bicephalous *Tegenaria atrica* and exposure of their embryos to temperature fluctuations. By exposing spider embryos to alternating temperatures of 14 and 32 °C for the first 10 days of embryonic development, we obtained eight two-headed individuals, subsequently divided into three groups according to morphological differences. We described in detail morphological abnormalities of the prosoma identified in members of each group. Histological examination confirmed a close relationship between morphological deformities and the brain structure of teratogenically changed spiders. The fusion of appendages (pedipalps and chalicerae) was accompanied by the fusion of corresponding ganglia. The absence of appendages (pedipalps) was accompanied by the absence of corresponding ganglia. This correlation may have resulted from previously impaired neuromere development which led to changes in the morphological structure of the prosoma. Since no deformities were identified in control animals, it can be concluded that bicephaly was caused by exposing embryos to alternating temperatures.

1. Introduction

There is abundant amount of information on morphological deformities of different body segments, appendages, and epithelia in many groups of arthropods including crustaceans (de Oliviera Dias, 1999; Fausto-Filho and de Costa, 1977; Fernandez et al., 2011; Feullassier et al., 2012; Follesa et al., 2008; Miličić et al., 2013; Spanó et al., 2003), insects (Asiain and Márquez, 2009; Ferreira, 2008, 2011; Reinert, 1999), myriapods (Leśniewska et al., 2009a,b; Mitić and Makarov, 2007; Mitić et al., 2011), and chelicerates (Ćurčić et al., 1983, 1991; Estrada-Peña, 2001; Kozel and Novak, 2013). Since many of these teratogenically modified animals were collected in the natural environment, the causes of their deformities remain unknown. The authors list several factors which could have been responsible for the changes during the embryonic period, but they leave them open for discussion.

It is much easier to analyze anomalies which are induced, i.e. develop after the administration of a certain dose of a selected teratogenic factor including humidity (Buczek, 2000), temperature (Jacuński, 1984, 2002a,b; Jacuński at al., 2004; Juberthie, 1962, 1963a,b, 1968; Napiórkowska and Templin, 2013), and various chemical compounds (Itow, 1979, 1980, 1982; Itow and Sekiguchi, 1979, 1980). Applied at a certain stage of embryonic development, these factors cause a range of morphological malformations of varying intensity. Bicephaly, the most complex and unique deformity of this kind, has been reported only on several occasions in anthropods, and in chelicerata in particular. Rempel (1954) described two embryos of the spider *Latrodectus mactans* (Fabr.) with a double prosoma, yet he did not mention the possible cause of this anomaly. Similarly, Matthiesen (1979) investigated an embryo of the scorpion *Tityus cambridgei* (Pocock) with a double front part of the prosoma, two sets of eyes, and four pedipalps. *Tegenaria atrica* affected by bicephaly have been studied by Mikulska and Jacuński (1970, 1971), who induced this anomaly by raising temperature during embryo incubation. Prosoma morphology of bicephalous *Tegenaria* was also analyzed in the subsequent studies by Jacuński and Templin (2003) and Templin et al., (2009). Varying temperatures applied by the authors caused not only a duplication of the front part of the prosoma but in some spiders, a number of additional anomalies, which significantly affected the morphology of this body part. The application of the same thermal method caused an even more severe deformity in one *Tegenaria* spider, described by Jacuński (1992) as tricephalous.

Although all of the above studies offer a detailed description of the external anatomy of bicephalous monsters, they fail to provide

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the analysis of their internal anatomy, especially of the central nervous system. Jacuński and Templin (1992) found two brains in bicephalous individuals. They provided only a general description of the brains and did not report their histological analysis. It is therefore unclear whether there were any additional changes (such as the fusion of cephalic ganglia) in these cases of bicephaly. Our study involved the examination of histological sections from *Tegenaria atrica* nymph II which had and additional head with a set of eyes and chelicerae near fovea on the dorsal side of the body on a fully formed prosoma (Napiórkowska et al., 2010). A nervous mass in the head functioned as the brain.

The application of the thermal method enabled us to obtain spiders with two fully formed heads or with one fully formed head and one partially formed, situated on the side. Since such anomalies can be accompanied by additional defects including the fusion of chelicerae and pedipalps, the structure of the central nervous system, particularly of the brain/brains, is likely to be altered. However, this topic has not yet been sufficiently explored. Before the experiment we assumed that the absence of an appendage/appendages would be accompanied by the absence of the corresponding ganglia in the brain, whereas the fusion of appendages, by the fusion of the corresponding ganglia. The experiment was aimed at demonstrating the relationship between deformities of the front part of the prosoma accompanied by changes in the brain structure in bicephalous *Tegenaria atrica* and exposure of their embryos to temperature fluctuations.

2. Materials and methods

The study involved specimens of *Tegenaria atrica* C.L.Koch (1843). It is a synanthropic spider species, common in Poland. Its breeding season is in autumn and winter. 91 sexually mature females and 31 males collected in August and September (2012–2014) near the towns of Chełmża and Toruń (Poland) were transported to the laboratory, where each spider was put into a separate glass container with a capacity of 250 cm^3. Spiders were kept in a dark room and offered the conditions optimal for the species, i.e. the temperature of 21–23 °C and relative humidity of 70% (Jacuński et al., 1994; Mikulska and Jacuński, 1968). Both males and females were fed twice a week larvae of *Tenebrio molitor* Linnaeus. Two weeks after the culture was established, a sexually mature male was introduced into the container with a female ready for fertilization. Because we collected fewer males than females, one male paired with several females; each male was thus the father of offspring of several mothers. The first cocoons were laid at the end of September. Embryos removed from the cocoons were counted and divided into two groups: the control group, maintained in conditions optimal for the embryonic development of this spider species (Jacuński and Wiśniewski, 1997), and the experimental group exposed to temperatures of 14 and 32 °C applied alternately every 12 h. The procedure continued for ten days, until the first metameres of the prosoma appeared on the germ band. Subsequently, all experimental embryos were incubated under the same conditions as the control ones. Hatching took place approximately 20 days after the eggs were laid. All control and experimental larvae (Vachon, 1957), sometimes alternatively called the “post-embryo” by some authors (Downes, 1987; Wolff and Hilbrant, 2011) were evaluated for developmental deformities. Specimens with anomalies of the front part of the prosoma were placed in separate containers and photographed. On the second day after hatching they were fixed in Bouin solution. 7 μm thick histological sections were prepared using the paraffin technique and stained with hematoxylin and eosin (Zawistowski, 1986).

3. Results

We obtained approx. 2500 embryos, half of which constituted a control group, where the mortality rate was around 5%. Larvae which emerged from eggs did not have any developmental abnormalities. There were 6 pairs of well-developed appendages on the prosoma of all the spiders in this group (Fig. 1A). The histological analysis of randomly selected individuals showed no changes in the structure of the nervous system, particularly in the brain (Fig. 1B).

The mortality rate among experimental embryos, exposed to alternating temperatures, was significantly higher and amounted to approx. 25%. 166 spiders in this group had various body deformities including the absence of legs or spinnerets (oligomely), the fusion of legs situated next to each other (heterosymely), bifurcation of legs (schistomely), the presence of additional legs (polymely), the fusion of legs situated on the opposite sides of the prosoma belonging to the same pair (symely), and the presence of two heads (bicephaly). Several individuals were affected by complex anomalies (a number of anomalies occurring simultaneously) (Table 1). Fig. 2A-C presents selected cases of anomalies obtained in this series of teratological experiments.

Bicephaly was identified in 8 larvae, divided into three groups according to the type of morphological defects. The first group consisted of two individuals which had two fully formed heads, each with two chelicerae (ch1, ch2 and ch3, ch4), two separate six-segmented pedipalps (pp1, pp2 and pp3, pp4) with separate gnathocoxae and two labia (Fig. 3A, B). The second group consisted of five individuals which had two pairs of chelicerae (ch1, ch2 and ch3, ch4) and two pairs of pedipalps, but the pedipalps situated

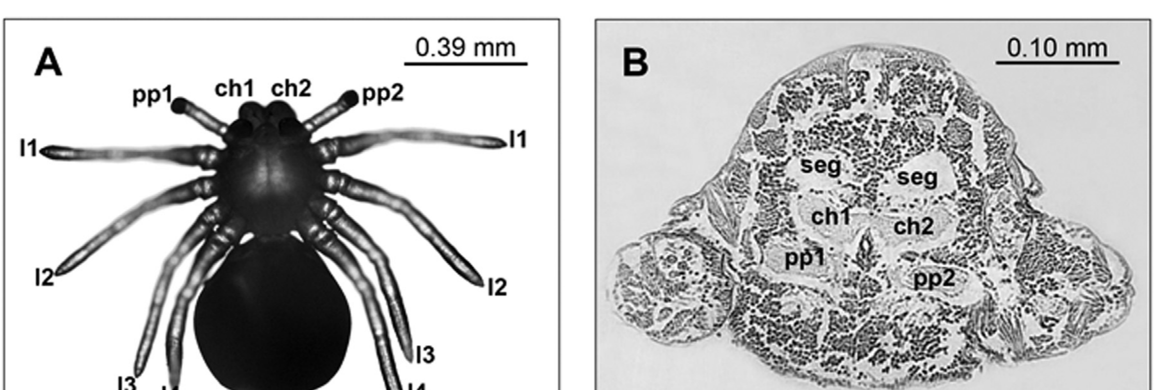

Fig. 1. A,B. A – External morpfology of *Tegenaria atrica* larva from the control group (*ventral view*): pp1, pp2 – pedipalps, ch1, ch2 – chelicerae, l1–l4 – walking legs; B – transversal section through the anterior part of the prosoma: ch1, ch2 – ganglia of chelicerae, pp1, pp2 – ganglia of pedipalps, seg – brain.