Teratological changes in the *Eratigena atrica* larvae obtained as a result of the application of alternating temperatures on spider embryos

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Commented [MT1]: There is no general agreement on terminology applied to different developmental stages in spiders. Therefore, describing the stage documented in this study as 'larvae' is acceptable and it has precedent (e.g. Vachon, 1957). But it may not be the best choice given what the term denotes in the context of insects. As Downes (1987) says: "a larva is understood by entomologists to be an actively feeding form so different from the adult … that it is separated from the latter by a metamorphosis. The spider 'larval' stage … differs not in gross form but only in developmental detail from the later instars, and is normally to be found not actively feeding but lying on its back and feebly waving its unsegmented legs." Downes' recommendation is to use the term 'postembryo' for this stage.
Since the authors have used 'larvae' in earlier papers, to remain consistent within themselves, they might retain 'larvae' and at the first mention of this word within the text make it evident that this is the stage produced by hatching of the embryo (egg) and that other terms are sometimes applied to this stage, including postembryo, first instar, and others as listed by Downes.

Commented [MT2]: Rather than saying 'in the *Eratigena atrica* larvae', I think it would be better to say 'in larvae of *Eratigena atrica*'.

Abstract

Spider embryonic development depends on several factors, including temperature. Under optimum thermal conditions embryogenesis proceeds undisturbed and embryo mortality is low. On the other hand, dramatic shifts in incubation temperature may cause a range of developmental defects in embryos. It has been confirmed in numerous laboratory experiments that abrupt temperature changes can be a powerful teratogenic factor. Changes in the external structure are frequently reflected in the internal anatomy, and above all, in the central nervous system. In the present teratological study, by exposing spider embryos to the temperatures of 14°C and 32°C, changed every 12 hours for the first 10 days of their development, we obtained 74 larvae with body deformities such as oligomely, heterosymely, schistomely, bicephaly, complex anomalies and others. We selected six spiders to describe and analyze their morphological changes. In one case, that of a~~i.e. in the~~ spider affected by polymely (the presence of a supernumerary appendage) combined with heterosymely (the fusion of walking legs), we also focused on the structure of the central nervous system. The analysis indicated that this complex anomaly was accompanied by only one change in the central nervous system: the presence of a supernumerary neuropil. Since no fusion of walking leg neuropils was observed, it was concluded that, in this instance, there was no relationship between the morphological deformity and the structure of the central nervous system.

Commented [MT3]: Maybe replace 'morphological deformity' with the more specific 'fusion of legs' to make the sentence clearer.

Introduction

Teratology is a relatively new field of knowledge, dating back to the early 20th century. Owing to advances in genetics, toxicology, molecular biology, animal testing, and research on living organisms-environment interactions, teratology has developed significantly in recent years (*Calado & dos Anjos Pires, 2018*). Currently there are many known teratogenic factors. Their teratogenicity has been confirmed in numerous experiments, which aid~~help~~ our understanding of both developmental defects and their mechanisms and normal processes occurring during embryogenesis (*Wilson, 1964*). According to the principles of teratology/developmental toxicology, toxins acting on embryos cause dysmorphogenesis when applied in a sufficient dose during a sensitive period in the development of a sensitive species. Though~~In spite of the fact that~~ in vitro studies can provide reliable means to assess the potency of teratogenic/toxic substances, there is still a need to use animal models to demonstrate their embryotoxicity (*Carvan III et al., 2004*). Invertebrates seem to be particularly useful for testing the toxicity/teratogenicity of different factors, including environmental ones. These animals occupy key positions in the food chain, in aquatic and terrestrial ecosystems, and some species or groups of species are found throughout the entire habitat. They have been used for decades in toxicity tests so they have an enormous potential to help identify environmental hazards. Invertebrates have a number of characteristics that facilitate their breeding including small size, high fertility rate and short lifespan. These factors, together with low purchasing cost, ensure relatively easy and very efficient application in laboratory testing (*Lagadici & Caquet, 1998*).

Arthropods, including spiders, in which~~with~~ the body is divided into the prosoma and opisthosoma, are considered to be excellent models for teratological research. Various teratogenic agents applied to spider embryos may cause deformities in both tagmata. Most

Commented [MT4]: I think early 19th century might be more accurate. I. Geoffroy Saint-Hilaire, for example, published his *Traité de Tératologie* in 1836 and his father E. Geoffroy Saint-Hilaire published *Considérations Générales sur les Monstres* in 1826, the former a substantial multi-volume treatise.

commonly, these defects are found on the prosoma and its appendages and are easy to detect. In addition, processing a histological specimen for examination, i.e. for an assessment of changes in the internal structure, is a straightforward task. The synanthropic spider *Eratigena atrica* (C.L. Koch) (previously *Tegenaria atrica*) from the family Agelenidae ~~family~~ has been widely used in teratology research. ~~An i~~Important features of this species are~~is~~ the relatively long breeding season in autumn/winter, high fertility rate and large embryo size. A number of experiments have been carried out to induce developmental deformities in this spider species (*Jacuński, 1969*; *Napiórkowska, Jacuński & Templin, 2010b*; *Napiórkowska, Napiórkowski & Templin, 2016a*; *Napiórkowska & Templin, 2017a; Napiórkowska & Templin, 2017b; Napiórkowska & Templin, 2018*).

I~~t is a known fact that i~~n the natural environment spider embryos are exposed to a range of factors which can affect their development, causing various morphological deformities. It has been confirmed in numerous laboratory experiments that abrupt temperature changes can be a powerful teratogenic factor (*Jacuński, 1971; Jacuński, 1984; Jacuński & Templin 2003; Napiórkowska, Jacuński & Templin, 2010a; Napiórkowska, Jacuński & Templin, 2010b; Napiórkowska, Napiórkowski & Templin, 2016a; Napiórkowska, Napiórkowski & Templin, 2016b*). The application of alternating temperatures (lower and higher than the optimum) during the early stages of embryonic development of *Eratigena atrica* led to a range of deformities of the prosoma and opisthosoma (*Jacuński, 1984*). Understandably, some of these changes prevented deformed individuals from going through successive stages of postembryogenesis. With seriously impaired locomotion, they were unable to hunt, feed, moult and reproduce. Numerous anomalies, including oligomely (absence of one or more appendages), symely (fusion of appendages of the same pair), schistomely (bifurcation of appendages), heterosymely (fusion of adjacent appendages), polymely (appearance of one or more additional appendages), bicephaly (presence of two heads), and so-called complex anomalies (several anomalies occurring simultaneously), have been identified in teratogenic studies (*Jacuński & Napiórkowska, 2000*; *Jacuński, Templin & Napiórkowska, 2005; Napiórkowska & Templin, 2013; Napiórkowska, Jacuński & Templin, 2007; Napiórkowska, Napiórkowski & Templin, 2015; Napiórkowska, Templin & Napiórkowski, 2013*). Some of them (oligomely) were observed with high frequency, others were quite rare (bicephaly) (*Jacuński, Templin & Napiórkowska, 2005; Templin, Jacuński & Napiórkowska, 2009*). In many instances, the description of morphological defects was followed by a histological analysis of deformed spiders. Particular attention was paid to the central nervous system (*Napiórkowska, Jacuński & Templin, 2010a; Napiórkowska, Jacuński & Templin, 2010b; Napiórkowska & Templin, 2017a; -Napiórkowska, Templin & Wołczuk, 2017*).

The structures of the digestive and nervous systems have been extensively analyzed in individuals with complex anomalies. The results indicated different changes depending on the anomaly (*Napiórkowska, Napiórkowski & Templin, -2015; Napiórkowska, Templin & Wołczuk, 2017*);~~. Moreover,~~ morphological deformities were not always reflected in the internal anatomy. Therefore, preparation of histology slides of individuals affected by new types of complex anomalies would facilitate the classification of the defects.

In the breeding season 2017/2018 the application of alternating temperatures during early embryogenesis of *Eratigena atrica* provided~~let~~ us with~~obtain a~~ new, interesting cases in the teratogenic material. Although this method has been used in teratology research on this spider species for years, it can still produce unpredictable results. These new, random anomalies are worth discussing in detail. Our study also emphasizes the power of temperature as a teratogenic agent, whose application in the laboratory may cause such extensive changes in spider anatomy and morphology that affected individuals are unable to express normal behaviour or develop a reproductive strategy. Therefore, the aim of the study was to show the diversity of anomalies in terms of morphological changes as well as, in one spider, anatomical

Commented [MT5]: Maybe replace 'changes' with 'internal effects'.

changes ~~(one spider)~~. In the latter case it was hypothesized that morphological changes were reflected in the structure of the central nervous system.

Material and methods

The teratological experiment involved embryos of *Eratigena atrica* (C.L.Koch, 1843). 24 sexually mature females and 17 males were collected in early autumn near the towns of Chełmża and Toruń (Poland) and 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 at the temperature of 21- 23˚C and relative humidity (RH) of 70%. Three weeks after the culture was established, males were introduced into containers with females ready for fertilization. First cocoons were laid after several weeks. Embryos removed from the cocoons were counted and divided into two groups: the eggs of each female were divided equally into two groups: experimental and control. The control group was kept at the temperature of 22˚C and ~~the humidity of~~ 70% RH, while~~and~~ the experimental group was exposed to temperatures of 14°C and 32˚C applied alternately every 12 hours. The procedure continued for ten days, until first metameres of the prosoma appeared on the germ band and the leg formation process began (embryo development was observed in paraffin oil; the chorion becomes transparent in paraffin oil ~~becomes transparent~~). Subsequently, all experimental embryos were incubated under the same conditions as the control ones. After hatching, all control and experimental larvae were examined for developmental deformities on the prosoma and opisthosoma. Deformed individuals were photographed, and one was subjected to histological analysis, in which 7-µm-thick -paraffin sections were stained with Mayer hematoxylin and eosin (Mayer's haemalum technique).

Commented [MT6]: In the context described in this sentence, I think it is insemination that occurs, rather than fertilization. I think fertilization does not occur until the time when eggs are being laid.

Commented [MT7]: Use of the word 'cocoons' raises the same concern that I expressed earlier with respect to the term 'larvae': a term primarily from entomology applied to something non-equivalent in araneology. In entomology, a cocoon is a construction in which a pupa develops and it is used by the same individual that produces it. True, a spider 'cocoon' also affords protection to the animals within. I just know that I have seen objections in the literature on multiple occasions to use of the terms 'cocoon' and 'larva' applied to spiders. Suitable replacements here include 'egg sac' and 'egg case'. Not critical; just something to consider.

Commented [MT8]: This can be said more concisely so that 'divided into two groups' is not repeated. For example, 'Embryos were removed from each egg sac, counted, and divided equally into two groups: experimental and control.'

Commented [MT9]: Also maintained at 70% RH?

Commented [MT10]: See comment above at title. If the authors prefer to retain 'larvae', they could modify this to say something like "...larvae (= postembryos of Downes (1987)) were examined..."

Results

In the breeding season 2017/18 we obtained approximately 6000 embryos, half of which constituted the control group. Individuals from this group were not affected by any developmental defects and the mortality rate was low (8%). In contrast, in the experimental group the mortality rate of embryos was much higher (37%). This group contained several larvae with teratogenic changes on the prosoma or opisthosoma. In total, 74 out of 1,900 larvae were affected by one of the following anomalies within the prosoma: oligomely, heterosymely, schistomely, bicephaly, complex anomaly and others. In the latter group were larvae with considerably shorter appendages of the prosoma, protuberances of different size and shape or anomalies in the spinning apparatus (Table 1). Several interesting cases selected for ~~the~~ analysis are presented in Figure 1.

The spider in Figure 1A was affected by bilateral oligomely and, apart from one pair of pedipalps (p), had only three pairs of walking legs (l1-l3). Additionally, it had a truncheon-shaped protuberance (a) in place of the left chelicera (dorsal view). A similar protuberance (a) developed in place of one pedipalp in the spider presented in Figure 1B. The remaining appendages on the prosoma, i.e. chelicerae (c), left pedipalp (p) (dorsal view) and walking legs (l1-l4) were well-developed and had the correct size and segmentation. In the spider in Figure 1C a complex anomaly was observed. The specimen had one chelicera (c), one pedipalp (p) and only two walking legs (l1 and l2) on the left side of the prosoma (ventral view). The right side of the prosoma was significantly changed. Behind the chelicera (c) there was a schistomelic pedipalp (p), with~~hose~~ one free end ~~was~~ much shorter and deformed. Behind the pedipalp there was also a short protuberance (a), widened in the middle, and only two walking legs (l1 and l2). A complex anomaly was also recognized in the spider in Figure 1D. This individual was affected by oligomely of walking legs on the left side of the prosoma

Commented [MT11]: If you know the exact number of embryos that were used, I would state that number.

Commented [MT12]: A small matter, but here and throughout the manuscript, including in the figures, if it is not too difficult to change, it seems like readers would have an easier time viewing a designation like L1-L3 rather than l1-l3, which is so close in appearance to 11-13.

Commented [TM13]: Double-check this: the figure caption for Fig. 1 says 1A is a ventral view (and it looks like a ventral view). If so, then the protuberance is actually in the position of the right chelicera, correct?

Commented [TM14]: Again, 'left' and 'right' seem backward to me. Fig. 1C is a ventral view, so the left side of the photo is the spider's right side, correct? If I have that correct, then, in these two sentences, where it says 'left', it should be 'right' and vice versa.

Commented [TM15]: Just out of curiosity, in the photo it looks like one end of this protuberance attaches to the prosomal trunk and the other attaches to L1 (like a by-pass on a highway map). Is that correct? If so, I think it would be worth mentioning since it is a type of anomaly I have not seen reported elsewhere.

Commented [TM16]: right

(ventral view), because behind the fully formed chelicera (c) and pedipalp (p) there were only three walking legs (l1-l3). On the right side of the prosoma the first walking leg (l1) was schistomelic. The bifurcation started at the tibia. The non-bifurcated part of the leg was thicker than usual, with distinct segmentation. The two free ends, which extended in opposite directions, were also distinctly segmented. Posterior to~~Behind~~ the schistomelic leg were three well-developed walking legs (l2-l4). Figures 1E and 1F show two ~~dramatically~~ different bicephalous specimens. One (Fig. 1E) had two equivalent heads with a double set of chelicerae (c) and pedipalps (p). Between the heads were two fully formed, separate walking legs (l). The other specimen (Fig. 1F) had two complete heads with chelicerae (c), pedipalps (p) and two walking legs (l/l) in between, fused from coxa to the patella. Both individuals also had a standard set of walking legs (l1-l4).

A histological analysis was performed on one individual affected by an anomaly that had not been previously recorded. The case seemed interesting both in terms of morphology and internal anatomy. As can be seen in Figure 2A the spider had a complex anomaly, i.e. polymely and heterosymely of the walking legs on the right side of the prosoma (dorsal view). Behind a well-formed, six-segmented pedipalp (p) was a very thick, significantly deformed appendage (a) with two free ends (l1; l2/3). Based on its unique appearance, it was assumed that it consisted of three walking legs, which would mean that five walking legs developed on this side of the prosoma (polymely). The last two legs (l4; l5) were well-developed with seven segments. The first three were heterosymelic. Two of them (l2/3) were fused over their entire length (total heterosymely). In addition, they were fused with the first leg (l1) along the coxae, trochanters, femurs, and patellas (partial heterosymely), which explains the presence of the two free distal ends: l1 and l2/3. The assumption was that the end l2/3 was much thicker because it was composed of the last three segments of the completely fused legs. The segmentation of this end was very indistinct, but its length was the same as that of the end of

Commented [TM17]: left

Commented [MT18]: They are different but 'dramatically different' seems too strong. It looks like the only substantial difference between the two specimens concerns the two walking legs that formed between the two heads: separate in 1E, fused proximally in 1F.

l1, which consisted of three ~~part~~segments. On the opposite side of the prosoma there was a set of properly formed~~built~~ appendages.

To verify our assumptions we prepared histology slides of the central nervous system of the investigated spider. There were no structural changes in the brain (Fig. 2B). However, in the ventral nerve cord (subesophageal ganglia) the number of leg neuropils was higher. Figure 2C shows the neuropils (n) of the ventral nerve cord in its middle part, with four separate walking leg neuropils (n1, n2, n4, n5) apart from the neuropil of the pedipalp (np) on the deformed right side. The last two on this side of the prosoma (n4, n5) were the neuropils of the well-formed~~built~~ walking legs ~~(~~l4 and l5~~)~~; the first two (n1, n2), those of the heterosymelic legs. Based on the location, n1 was assumed to be the neuropil of the leg whose distal end was marked as l1 and one of the two legs which were completely fused (end l2/3, neuropil n2). The ventral nerve cord contained one abnormal~~more~~ additional neuropil, moved to the ventral side (n3) (Fig. 2D). ~~A h~~Histological analysis indicated that it belonged to the second leg of the fused complex, whose end was marked as l2/3. No fusion of the leg neuropils was observed.

Discussion

For the present study, 1,900 larvae that left their eggshells after ~~the~~ exposure to the teratogenic agent (alternating sub- and supra-optimal temperatures) were examined for developmental deformities. 74 individuals, i.e. 3.9%, had body defects. Assuming that thermal shocks applied during spider embryogenesis are a potent teratogen, the number seems low. There may be several possible causes of such a low frequency of deformities. First~~ of all~~, effective repair processes at every stage of embryo development may eliminate errors that occur during morphogenesis. Second~~In addition~~, spiders, which are ectothermic animals, must be relatively resistant to abrupt temperature changes. This would also apply to spider embryos, although their mortality was relatively high (37%).

Commented [MT19]: Maybe replace ‘apart from’ with ‘in addition to’.

Commented [MT20]: This makes it sound like you are saying n1 is the neuropil for both L1 and either L2 or L3. But I think you mean to say that n1 is neuropil for L1, and n2 is neuropil for one of the totally fused L2/L3. Is that correct?

Commented [MT21]: Maybe replace ‘moved to the ventral side’ with ‘displaced ventrally’.

Commented [MT22]: ‘several’ generally implies more than 2. Since only two reasons are given, I would use the word ‘multiple’.

Commented [MT23]: I think the word ‘reasons’ is more appropriate here, especially since it sounds odd to describe ectothermy as a ‘cause’ for infrequent anomalies.

Oligomelic individuals were the most numerous in the teratological material (over 50%), which coincides with the results of previous studies (*Jacuński, 1984; Napiórkowska, Napiórkowski & Templin, 2016b*). A relatively large group of larvae (23%) had deformities classified as 'Others' in Table 1, followed by larvae with so-called complex anomalies (15%). Since many deformities obtained as a result of the application of temperature changes during embryonic development of *Eratigena atrica* ~~-~~have~~d~~ already been described (e. g. *Napiórkowska, Napiórkowski & Templin, 2015; Napiórkowska, Napiórkowski & Templin, 2016a*), we focused on those that were encountered for the first time. Based on the previous observations it can be predicted that new surprising changes may occur when developmental processes are disturbed by temperature shocks. Every year novel~~new~~ body deformities are registered in teratological experiments.

In one~~ particular~~ case we analyzed not only deformities of the walking legs on the right side of the prosoma but also the structure of the central nervous system.

The nature of this malformation suggests two processes: the formation of an additional leg (polymely) and the fusion of three walking legs (heterosymely). Only the polymely was reflected in the central nervous system: an additional walking leg neuropil was~~could be~~ found in the ventral nerve cord, but neuropil fusion was not observed. Therefore, on the right side of the ventral nerve cord there were five walking leg neuropils and one of them was shifted to the ventral side. According to *Jacuński* (*1984*), an additional leg, not developed during normal~~in a regular~~ ontogenesis, is associated with the appearance of an additional half of a metamere (and thus of a neuromere) on the germ band. This suggests that all polymelic legs should have their ganglia, as has been~~the~~ observed in~~ation that has been confirmed by~~ numerous studies (e.g. *Napiórkowska, Napiórkowski & Templin, 2015*). However, two different scenarios have been observed in instances of this anomaly: 1) an increased number of ganglia and their fusion, despite the absence of fused legs, 2) an increased number of

Commented [MT24]: Did your histological sections indicate any fusion between n2 and n3 along the dorsal-ventral axis? You might specifically state what you found in this regard.

Commented [MT25]: For clarity, consider replacing 'this anomaly' with 'polymely' or 'leg polymely'.

ganglia and no leg or ganglia fusions. The spatial location of the ganglia is another issue. In the majority of cases the ganglia (neuropils), including the supernumerary ones, were located in one plane (*Napiórkowska, Templin & Wołczuk, 2017*). In several individuals the ganglia were shifted to the dorsal or ventral side (*Napiórkowska, Napiórkowski & Templin, 2015*). It is therefore important to understand the causes of these shifts. First, they may be induced by changes in the genes responsible for the formation of the anterior-posterior and dorsal-ventral axes, which determine the location of all internal organs and structures. The arrangement of these organs, established at the beginning of embryonic development, may~~can~~ be disturbed by the application of thermal shock to the embryo. Another explanation could be space limitation: since the size and symmetry of the prosoma does not change (despite the presence of an additional leg and additional half of a~~the~~ neuromere) an additional ganglion has to be "pushed"~~, to the~~ ventrally or dorsally~~, side~~ in order to fit into a limited volume of the prosoma. In the investigated spider the heterosymely of walking legs was not associated with the fusion of their ganglia (neuropils), although it seems logical that it ~~sh~~could have been reflected in the central nervous system. This would have suggested a certain hierarchy between segmental structures, with the fusion of ganglia leading to the fusion of the corresponding legs. Such a situation was observed in bicephalous *E. atrica* whose chelicerae and pedipalps were completely fused (*Napiórkowska et al., 2016c*). However, in the vast majority of cases, heterosymely has not been accompanied by the fusion of leg ganglia (*Napiórkowska, Napiórkowski & Templin, 2015; Napiórkowska, Templin & Napiórkowski, 2013*). This indicates that the fusion of walking legs may result from the fusion of the developing leg buds, caused by the exposure of an early embryo to thermal shock. In other words, thermal shock may affect the developing leg buds, but not necessarily leg ganglia. Temperature changes applied during embryo incubation may or may not affect various elements of~~ the~~ repeated structures, such as ganglia or legs. The consequences depend on the intensity of the

Commented [MT26]: Please provide at least one citation for each of these two scenarios.

Commented [MT27]: Is the inverse also possible? That is, could fused legs lead to fused ganglia?

Commented [MT28]: Maybe 'serial' instead of 'repeated' thermal shock and time of its application. From this point of view, an application of a teratogen in early stages of embryogenesis may cause more profound changes than its later application and a range of effects may be expected. Moreover, since morphological defects are not always reflected in the central nervous system (an example of which is the investigated *E. atrica*), teratological studies should not be limited to deformity descriptions but should also focus on internal anatomical examination, including that of the central nervous system. This type of research has already been conducted on spiders and other arthropods (*Harzsch, Benton & Beltz, 2000; Jacuński, Templin & Napiórkowska, 2005; Scholtz, Ng & Moore, 2014*).

A certain analogy can be seen between the investigated *E. atrica* and a deformed pycnogonid *Pycnogonum litorale* described by Scholtz & Brenneis (*2016*). In the latter, an extensive deformity resulted from a mechanical, unintentional injury to the region between the second and third walking leg. After several months the sea spider developed an extra leg on the right side of the prosoma -partially fused with other legs. In this case, the supernumerary leg did not have an associated ganglion although it did, like the other legs, contain~~have~~ a midgut diverticulum~~a~~ and a branch of ovary. Scholtz & Brenneis (*2016*) explained the anomaly using the "boundary model" proposed by Meinhardt in the 1980s and confirmed later by molecular data. This model hypothesized the division of each body segment into at least three cellular compartments, designated S, A and P, along the antero-posterior axis~~assumes that limbs and other lateral branches of the body axis are formed at a boundary where cell populations with at least three different states meet~~ (*Meinhardt, 1986*). Two of these were known ~~F~~from *Drosophila* research, with~~it was known that~~ each segment comprised~~s~~ of transverse cell populations with an anterior or/and a posterior fate, the A and P compartments, respectively, which lie strictly separated but adjacent to each other (*Martinez-Arias & Lawrence, 1985*). Meinhardt's~~oreover, the~~ model further hypothesized~~implies~~ that, perpendicular to each A-P

Commented [MT29]: You might also mention what stage the pycnogonid was in when the injury occurred. Was it an embryo, juvenile, etc?

Commented [MT30]: Would the word 'supported' be more appropriate than 'confirmed'? That is, how well-established is it that the Meinhardt model is correct? 'confirmed' suggests a high degree of certainty.

Commented [MT31]: This statement does not seem correct to me. As you say three sentences later, limbs form at the AP-DV intersection in Meinhardt's model. The three different states as proposed by Meinhardt, A, P, and S, do not all meet where limbs form: S, along with P, is involved in determining where segment borders form, but it is not directly involved with limb location. Instead, the A-P boundary interacts with perpendicular D-V boundaries for limb initiation.

Commented [MT32]: Can a population have both an anterior and posterior fate? Or is it one or the other, A or P? My very limited understanding is that it is one or the other, in which case this 'and' can be deleted, but I could be mistaken.

border, there is a longitudinal boundary separating dorsal (D) and ventral (V) cells on either lateral side of the embryo (*Meinhardt, 1986*). At intersections~~In the contact zone~~ between A-P~~anterior and posterior cells~~ and ~~the~~ D-V~~dorso-ventral~~ borders, the formation of limb buds is initiated. If the cells of an S compartment~~with the third cell state between adjacent segment~~ are removed, the P~~posterior~~ cells of an~~the~~ anterior segment form a contact zone with the A~~anterior~~ cells of the more posterior segment and an additional leg is formed. This model could also be used to explain the formation of an additional leg in the investigated *E. atrica*. Furthermore, molecular analysis might help explain the mechanisms of morphological defects in this spider. Many researchers, including -Pechmann et al. (*2011*) and Khadjeh et al. (*2012*), have successfully conducted such studies on spiders.

All developmental defects, both those that are caused by some complex regenerative processes and those that are caused by teratogenic factors~~,~~ (e.g. alternating temperatures applied during early embryogenesis), can contribute to a better understanding of developmental mechanisms in invertebrates. In addition, they indicate morphological capabilities and developmental potential of organisms, not displayed under normal conditions. Teratological experiments based on temperature seem justified in view of current weather anomalies. Sudden temperature changes observed nowadays can affect embryos, causing damage that may not be subject to spontaneous repair processes. As a consequence, a higher number of deformed animals may be found in the natural environment. Observation of spiders, commonly found near human settlements, can provide abundant evidence of adverse environmental impacts.

In conclusion, our results suggest that temperature changes during embryonic development of animals can cause various deformities in their body structure. Our findings provide additional evidence that morphological defects are not always reflected in the central nervous system (an example of which is the investigated *E. atrica*). Therefore, teratological studies should not be limited to describing external features of deformed individuals, but should also involve analyzing their internal organs, including the CNS.

Declaration of competing interest

The authors declare they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Figure 1

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Eratigena atrica larvae with teratogenic changes.

A ventral view: larva with bilateral oligomely and a protuberance in place of the right~~left~~ chelicera; **B** dorsal view: larva with a protuberance in place of the right pedipalp; **C** ventral view: larva with oligomely of the walking legs, schistomely of the left~~right~~ pedipalp, and a protuberance between the pedipalp and walking leg; **D** ventral view: larva with oligomely of the walking legs on the right~~left~~ side of the prosoma and schistomely of the first left~~right~~ walking leg; **E** dorsal view: bicephalous larva with additional, well-developed walking legs between two heads; **F** ventral view: bicephalous larva with additional, partially fused walking legs between two heads; a, protuberance; c, chelicera; l, l/l, l1-l4, walking legs, p, pedipalp

Figure 2

Eratigena atrica larva with complex anomaly.

A dorsal view: a, deformed appendage;~~,~~ l1, l2/3, free ends of fused walking legs;~~,~~ p, pedipalps;~~,~~ ~~l,~~ l1-l5, walking legs; **B**-**D** horizontal sections through the prosoma, brain (**B**) and ventral nerve cord (**C**, **D**) (right side abnormal~~changed~~, left~~opposite~~ side normal~~correct~~): a, fused~~common~~ part of the legs;~~,~~ n1, n2 (**C**) and n3 (**D**), neuropils of heterosymelic legs;~~,~~ n1-n4, n~~g~~5, neuropils of the well-formed~~built~~ walking legs;~~,~~ np, neuropils of pedipalps;~~,~~ n, neuropil.

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