ORAL PRESENTATIONS

Structure of micronuclei in the germ line cells of *Pelophylax esculentus*

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European water frogs are represented in Poland by two parental taxons, *Pelophylax lessonae* and *Pelophylax ridibundus*, and their natural hybrid *Pelophylax esculentus*. The hybrid can reproduce in a special manner called hybridogenesis, in which one of the parental genomes is eliminated from the germ line, and the second one is transmitted to the gametes. Ovarian development in larval stages of the parental species is well described, and before metamorphosis the gonad is filled with diplotene oocytes. However, the maturation of ovary in hybrids is delayed at the same somatic developmental stage. The cortex of ovary is formed by primary oogonia representing mitotic cells, as well as nests of meiotic oocytes, whereas diplotene oocytes are very rare. The nuclei in oogonia are often accompanied by micronuclei (MN), the spherical fragments of chromatin, 1-3 μm in diameter, surrounded by nuclear envelope (NE). The examination of NE structure and chromatin integrity in primary oogonia could elucidate the fate of micronuclei. If we assume that micronuclei are carrying the excluded genome we should expect the features of degradation processes in their structure.

Immunofluorescence studies were performed on *P. esculentus* ovaries collected at 39-45 Gosner stages of metamorphic climax. In tissue sections, the expression of nuclear envelope antigens, lamin B1 and F/G repeats nucleoporins was examined. Active caspase-3 and in situ TUNEL technique were used for apoptosis detection. Imaging was done with Olympus FV1000 confocal microscope. 3D reconstructions of NE using Imaris 6.2.1 software were used for visualization of the cellular structure.

Immunofluorescence analysis demonstrated that oogonial cells have two types of micronuclei. MN encapsulated by nuclear membrane including nucleoporins may represent the early stage of life after budding of the chromatin from the interphase nucleus or the lagging chromosomes existing in separate compartment after mitosis. The lack of nuclear pores in the second class of MN may precede nuclear envelope collapse followed by chromatin degradation in MN, saving main nucleus intact, as previously demonstrated in human cancer cell lines. Still, we need more experimental data about the apoptotic status of gonial cells harboring micronuclei. We suggest that cellular abnormalities observed in oogonial cells may be responsible for delayed gonad maturation in hybrid species.

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Characters of *Viola banksii* an endemic species of Australian *Erpetion* (Banks) W. Becker section of the genus *Viola* L. (Violaceae)

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The section *Erpetion* of the genus *Viola* L. is restricted to eastern Australia and Tasmania, it is high polyploid group (10x estimated ploidy) of 11–18 species. The crown group of this sect. has been estimated to ~5 million years old. The closest relative of sect. *Erpetion* is sect. *Chilenium* W.Becker distributed in southern South America (Marcussen et al., 2015). *Viola banksii* K.R. Thiele & Prober (2003) was recently recognized as a separate species within the section.

We analyzed embryological, micromorphological and anatomical characters of flowers and vegetative organs (primary root, stolon, petiole, peduncle, leaf blade), estimated chromosome number and genome size of *V. banksii* collected at different localities of east coast of Australia. *Viola banksii* is perennial, stemless, high polyploid ($2n=50$) with small genome size (2C=1.27 pg). It develops exclusively spurless chasmogamous (CH) flowers. The frequency of mature seeds in one capsule ranges from 7–60 (mean 20) with quite a few non-developed ovules per fruit. Our observation clearly showed that plants are self-compatible because in lack of pollinators set seeds. Pollen is not heteromorphic, 3-aperturate and highly viable (over 90%). The style of the pistil is straight, not head-like, glabrous, stigma tapering from the style. Two of five filamentless stamens have protuberance along the anther connective not connected with the vascular bundle of the anther. Their role as nectaries is disputable because there is lack of stomata, pores and cracks in cuticle layer in their epidermis through which a nectar may leak out. Anatropous, crassinucellate, bitegmic ovules, Polygonum type of female gametophyte development, suspensorless embryo of Asterad type and nuclear endosperm are similar as in other sections of the genus. *V. banksii* grows in shade, in moist, well-drained soils what is reflected in anatomy of its organs. There is a lack of sub-epidermal collenchyma layers in all aerial parts, leaves are amphistomatic with mean number of stomata on 1 mm^2 on abaxial epidermis 98 and 3 on adaxial epidermis, leaf epidermal cells are large with thin cell walls and cuticle layer, in vascular bundles xylem is not rich in vessels, primary roots are diarchic. It was difficult to find a unique traits of the *Erpetion* section based on known data of not related Northern Hemisphere sections e.g., *Viola*, *Melanium* Ging. and *Sclerosium* W. Becker which have been extensively studied. Anatomical characters when considered as useful in taxonomy should be analyzed with caution taking into account ecological preference of plants. Embryological traits are conservative, the main differences are in flower microstructure.

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Nanodiamonds as carriers of the insect gonadoinhibitory hormones

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Our previous studies have shown that injection of the insect peptide hormone, Neb-colloostatin (SIV-PLGLPVPIGPIVVGPR) induces atresia in the *Tenebrio molitor* ovary. The hormone caused degeneration of mealworm follicles includes changes in morphology and viability of follicular cells, and oosorption as a consequence of these changes (Czarniewska et al., 2014).

At present we suggest the use of nanodiamonds as carriers of the Neb-colloostatin through an insect cuticle due to the unique chemical and biological properties of this nanoparticle. Nanodiamonds and a complex of nanodiamonds and Neb-colloostatin after their administration on the *Tenebrio* cuticle inhibit ovarian development and terminal oocyte maturation of mated females during their first reproductive cycle. The application of nanodiamonds and nanodiamond-*Neb*-colloostatin complex also causes a delay to the first ovulation and oviposition as well as a reduction of the number of eggs in the first 7 days of the oviposition period. Moreover, during the first 14 days of the oviposition period, these molecules also reduce the number of hatched larvae from eggs laid by the females treated with these compounds.

The possibility of incorporation of nanodiamonds into body of insects has opened the way for investigation of their potential applications as carriers of gonadoinhibitory hormones to limit the number of harmful insects in the environment.

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Functional orthologs of human small heat shock protein HSPB8: *Drosophila melanogaster* Hsp67Bc and *Danio rerio* HSPB8

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Small heat shock proteins (sHSPs; HSPB) are ubiquitous proteins present in both vertebrates as well as invertebrates. Their most prominent activity is preventing, in an ATP-independent manner, the irreversible aggregation of various non-native proteins that may cause in the form of potentially pathogenic aggregates. During the stress response, sHSPs form the first line of defence against protein aggregation.

The sHSPs are involved in cell development, and the processes associated with aging, they are engaged in the regulation of apoptosis and influence on the architecture of the cytoskeleton and autophagy. Mutation in genes coding for these proteins are implicated in different myopathy- and neuropathy- associated diseases such as Charcot-Marie-Tooth syndrome.

To check functional similarity of both human HSPB8 orthologs *Drosophila melanogaster* Hsp67Bc and *Danio rerio* HSPB8 we have conducted series of studies including: analyses of protein distribution, knock down experiments. Our research were performed on these model organisms.

Our findings confirm that *D. melanogaster* Hsp67Bc, despite little sequence similarity, this protein seems to be a nearest functional orthologue of human HSPB8. During our studies, we confirmed neural (within the central nervous system) localization of the Hsp67Bc in embryos. As the development proceeds (in larval stages) Hsp67Bc persists neural localization with a slight increase at neuromuscular junctions (NMJ) and additionally shows also muscle distribution.

To verify Hsp67Bc function, we knocked-down it by muscle- and neural specific RNAi expression. Detailed research of both lines including analyses of arborization, number and size of the synaptic buttons revealed the altered morphology of NMJ. This data implies the involvement of Hsp67Bc in NMJ formation and development.

In the course of our research on the zebrafish HSPB8 function, we have shown that embryos reveal increased HSPB8 gene expression under heat shock conditions. It may suggest that HSPB8 can play a role as chaperone protein in zebrafish. We were also able to demonstrate that in normal conditions HSPB8 localizes in Z and M line whereas heat shock disturbs its distribution.

We were also interested in checking HSPB8 involvement in the zebrafish development. To do this, we have prepared knock down experiments by the used the morpholino antisense oligomers which block translation of mRNA. Morphants showed abnormalities in the tail length and muscle structure what may suggest HSPB8 involvement in the proper development of skeleton muscles in zebrafish.

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Morphological, histological and ultrastructural aspects of *Daucus carota* (L.) somatic embryogenesis

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Somatic embryogenesis (SE), a process in which somatic cells develop into embryos, is a good model for analyzing the mechanisms underlying zygotic embryogenesis and the plasticity of plant cells including their totipotency (Kurczynska et al., 2012).

In vitro cultures, where SE is easily induced (Steward et al., 1958), were applied for the studies upon the changes in symplast (Wrobel et al., 2011) and apoplast (Sala et al., 2012) during this process within the explant cells and in the somatic embryos. SE is a good model for analysis the transition of somatic cells to an embryogenic state, and somatic embryos are postulated to resemble most of the zygotic embryos features. The aim of presented studies was the analysis of morphological, histological and ultrastructural features of explant cells and somatic embryos of *Daucus carota* L., with special attention paid to the chemical composition of cell walls and exchange of information through plasmodesmata.

Obtained results showed that: 1/symplasmic communication between explant cells changes during the SE process and further somatic embryos development, 2/there is a correlation between the presence of pectic and AGP epitopes and the changes in the explant's cells fate and during somatic embryos development. Furthermore, the features of explant cells and somatic embryos, including the plasmodesmata occurrence, were determined on the ultrastructural level.

Our results will be discussed in relation to current knowledge, concerning the similarities and differences between zygotic and somatic embryos.

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Structure and ultrastructure of ovaries in two lizard species – slow worm *Anguis fragilis* L. and sand lizard *Lacerta agilis* L.*

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Ovarian follicular growth in reptilian species, as in all vertebrates is a crucial reproductive process of females. Thus, the identification and definition of morphological changes in the reptilian oocyte, and its peripheral structures during the female reproductive cycle are important for understanding the evolution of oogenesis. During annual cycles ovaries of reptiles change dramatically in shape and size according to the stage of the reproductive cycle, and the number and sequence of developmental stages of follicles. The aim of this study was to investigate structure and ultrastructure of ovaries after eggs laying in two national species of lizard – slow worm *Anguis fragilis* L. and sand lizard *Lacerta agilis* L. In this research standard methods for light and electron microscopy were used. The results of this study showed that the follicular epithelium surrounding oocytes maintains a polymorphic and multilayered organization. Ovaries of both species show the following architecture of three types of cells: intermediate, small and pyriform one. The small cells of the granulosa are cuboidal and show no obvious specializations. They have more or less oval nucleus containing numerous nuclear bodies with fibrous shells. Chromatin is condensed at the nuclear envelope and around the fibrilo-granular nucleolus. Intermediate cells are round and much larger than the small ones. They contain spherical nucleus with diffused chromatin and few nuclear bodies. The pyriform cells are the largest cells from granulosa. They can be recognized by an elongated apex contacting with the oocyte through bridges in zona pellucida. They are regularly distributed between small and intermediate cells, inside the multilayered follicular epithelium. Pyriform cells possess large spherical nucleus with diffused chromatin and prominent nucleolus very rich in granules.

*All specimens used in experiment were captured according to Polish legal regulations concerning wild species protection (Dz.U. nr 2 poz. 11 z 1984 r., Dz.U. nr 114 poz. 492 z 1991 r.). Approvals for performing studies on protected species were obtained from the Regional Directorate of Environmental Protection in Katowice (WPN.6401.72.2014.MS) and Wrocław (WPN.6401.99.2014.MK). The slow worm *Anguis fragilis* L. and sand lizard *Lacerta agilis* L. are not included in Washington Convention of 1973, ratified by Poland in 1991.

The vomeronasal organ embryonic development in the grass snake, *Natrix natrix*[*] (Squamata: Natricidae)

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In comparison to the olfactory system the vomeronasal system tends to be sensitive to less-volatile molecules. The function of the vomerolfaction is considered in the context of: prey location and discrimination, avoidance of predators and reproduction due to pheromonal communication. Squamate reptiles are characterized by well developed vomeronasal organ. There is no connection between vomeronasal organ and the nasal cavity in adults. A very narrow duct of the organ enters to the oral cavity through the palate anterior to the choana. Snakes are vomerolfaction specialists. Members of this clade typically have sensory epithelial columns, complete superficial palate associated with absence of choanal grooves and deeply forked tongue which allows to compare chemical signals from two different points without head movements. In our study we used light and scanning electron microscopy to describe development of vomeronasal organ in embryos of the grass snake (*Natrix natrix*). The age of embryos was calculated using the table of species development (Rupik, 2002). The material was fixed in 10% formalin solution or Bouin fluid. In order to light microscopy the material was subsequently embedded in paraffin and sectioned into serial transversal or sagittal sections. The sections for review were stained with H&E and azan. On the basis of these sections 3-dimensional reconstructions of the vomeronasal organ and associated structures were made. Our results showed development of: gross anatomy of the organ, vomeronasal epitlhelium and the cupola Jacobsoni. Additionally, we were able to describe the vomeronasal organ in relation to the nasal cavity and the nasolacrimal duct.

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Application of the genomic in situ hybridization (GISH): a case study of water frog hybrids of the *Pelophylax esculentus complex* (Ranidae)

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Genomic *in situ* hybridization (GISH) is one of the most important cytogenetic tools utilized in hybrid organisms. It enables to visualize, identify and thus distinguish between whole chromosomes (and their segments) of different parental genomes, evaluate karyotype characterization, interphase genomic organization and the degree of chromosomal rearrangements, as well as the degree of introgression in interspecific hybrids (alloploids) and polyploid organisms.

This method is based on the complementary pairing of highly and moderately repetitive sequences. Whole genomic DNA probes from one of the parental species (developed in Nick Translation process and labeled directly or indirectly with fluorochromes) hybridize with the target chromosome metaphase plates or interphase nuclei fixed on slide preparations. To improve the resolution and according to the phylogenetic distance and relationship, it is advisable to use the blocking DNA of the opposite parental species that competes with the probe. The results are subsequently analyzed using fluorescence microscopy.

Pelophylax esculentus complex consists of two parental species that reproduce sexually (*P. ridibundus* and *P. lessonae*) and their interspecific, hybridogenetically reproducing hybrid with hemiclonal heredity (*P. esculentus*). Hybridogenesis is a modification of gametogenesis. During that process one of the parental genomes is excluded from the germ cell line before the onset of meiosis and the remaining one is endoreplicated and then transmitted clonally into gametes.

The aim of our study is to examine the genome elimination process using GISH. We draw attention to genomic content of the germ cell line (diploid as well as triploid specimens) both in various developmental stages of tadpoles (including primordial germ and spermatogonial cells) and adult males. As a result, we want to point out exact stages in which genome is rejected, demonstrate whether it takes place only once in a life span or not, and show whether primary spermatogonial cells in adults possess homo or heterogenomic DNA content.

Our preliminary studies demonstrate that appropriate probe:blocking DNA ratio enables us to differentiate between parental genomes in hybrid's cell material being under investigation.

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Transovarial transmission of symbiotic microorganisms in leafhoppers from subfamily Deltocephalinae (Insecta, Hemiptera)

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Deltocephalinae is the largest subfamily within the family Cicadellidae (Insecta, Hemiptera). This group includes many common species, crop pests and vectors of plant diseases. These insects due to ingested food (mostly xylem sap low in essential amino acids) live in association with symbionts inhabiting their body (Baumann, 2005). Recent molecular studies (Takiya et al., 2006; Kobiałka et al., 2015) have shown that the symbiotic systems of Cicadomorpha are much more complex than previously thought. These leafhoppers are usually inhabited by two types of symbiotic microogranisms (e.g. *Sulcia muelleri* and *Nasuia deltocephalinicola* in the subfamily Deltocephalinae) which supply essential amino acids to their host. For this reason they have been named "coprimary symbionts" (Takiya et al., 2006). All "coprimary symbionts" are transovarially (vertically) transmitted between generations.

Conducted studies indicated that in the Deltocephalinae leafhoppers, the mode of transmission from one generation to the next seems to be uniform. In mature females, both types of bacteria – *Sulcia* and *Nasuia* – leave the bacteriocytes and accumulate around the follicular epithelium near the posterior pole of terminal oocytes at the stage of advanced vitellogenesis. The symbionts migrate across the follicular cells and gather in their cytoplasm. Next, the symbiotic bacteria start to accumulate in the perivitelline space. Finally, they form "a symbiont ball" in the deep depression of the oolemma.

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A new look at the Male Germ Unit (MGU) – epigenetic study

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Structural connection between vegetative nucleus and generative/sperm cells (Male Germ Unit) in gametophyte of flowering plants have been described in the 80's of last century (McCue et al. 2011). Nowadays MGU is increasingly considered not only as a structural unit but also as a functional one (Slotkin et al., 2009; McCue et al., 2011).

The aim of the study was an analysis of epigenetic modifications in the cells of mature pollen grains and the growing pollen tube of hyacinth. Using the immunocytochemical methods the level and distribution of selected markers of heterochromatin – DNA methylation (5metC) and histone deacetylase 1 (HDT1), and euchromatin – histone H4 acetylation (acH4), were investigated.

Our studies revealed that during germination and pollen tube growth in the vegetative and generative nucleus, and after division of generative cell also in sperm nuclei, epigenetic changes take place. This changes can be correlated with differences in transcriptional activity of those cells (Zienkiewicz et al., 2008, Zienkiewicz et al., 2011).

Especially interesting have proved to be chromatin areas of vegetative and generative nuclei, which were located in close proximity in the MGU in the growing pollen tube. The analysis of serial optical sections of MGU in confocal microscopy revealed that in chromatin of this region the decrease in DNA methylation, increase in histone acetylation as well as differences in the level and distribution of HDT1 were present. Those dynamic changes in the level and pattern of epigenetic markers indicate that changes in chromatin activity can occur in this area. Moreover, we suggest that genes specifically expressed during pollen tube growth could be located in this area of chromatin in both vegetative and generative nucleus. This observations give a new look at the role of MGU, which could be not only a structural but also a functional unit, which enables communication between male germ cells.

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Unique features of myogenesis in snakes – muscle growth and differentiation

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The somites in a grass snake (*Natrix natrix*) differentiate from unsegmented paraxial mesoderm in a rostral-caudal gradient. The somites are epithelial structures forming vesicles with centrally located somitocoel. Epithelial, cortical cells of somites express transcription factor-Pax3 protein (a marker of muscle progenitor cells). Further, somites differentiate into the sclerotome and dermomyotome. The myotome of *N. natrix* and Egyptian cobra (*Naja haje*) is composed of mononucleated myotubes. Among the multinucleated myotubes some mononucleated cells are present. These cells are believed to participate in muscles growth (both hypertrophy and hyperplasy).

Studies on *N. natrix* and *N. haje* myogenesis reveal some unique features of trunk muscles development. Our research shows two classes of skeletal muscles. The first class is composed of the typical muscle fibers, with myofibrils equally distributed in the sarcoplasm. The light and TEM analysis reveal significant differences in the second class of muscle fibers. The second class of muscle fibers is characterized by tightly paced myofibrils surrounded centrally located nucleus accompanied by numerous vesicles of different diameters. The histochemical and TEM analysis show the presence of lipid droplets in those fibers. Furthermore, the immunocytochemical analysis reveals that lipid droplets are observed only in slow/red muscles.

The phenomenon of lipid droplets in slow/red muscles was for the first time described during *N. haje* (Kannonn et al., 2016) and *N. natrix* myogenesis. Our results confirm the hypothesis that during trunk muscles development of snakes some of the muscle fibers are capable of lipid droplets storage as the most economical form of storing energy essential during hibernation.

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Mitochondria activity and distribution during spermatogenesis in the earthworm *Dendrobaena veneta* male germ-cell clusters

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Mitochondria are organelles that produce energy that can be found in almost every eukaryotic cell. It is known that the cells of different tissues have different energy demands. Numerous studies have shown that there is a correlation between the number of mitochondria and cell metabolism.

Mitochondria also play a very important role during gamete development. In oogenesis, mitochondria must be strictly selected because the oocyte will be the sole source of those organelles for every cell in the future organism. In spermatogenesis mitochondria, are crucial for sperm movement and its ability to fertilize the oocyte. The important role of mitochondria in gametes has led researchers to undertake studies focused on the metabolism of mitochondria and its segregation in germ-line cells. Results of these studies have shown that germ-line cells have a higher level of mitochondria density then somatic cells. One possible explanation for this result was proposed by Kirkwood and Holliday in their "disposable soma theory". This theory states that all of the processes that take place in germ-line cells must be very precise because they are crucial in order to avoid the accumulation of defective molecules that could negatively affect future generations. This theory appears to be true for the mitochondria in germ-line cells.

In order to distinguish active/inactive mitochondria in fluorescent microscopy, it is necessary to use special fluorescent dyes. The most frequently used are Mitotracker, DiOC6 and JC-1. The best results can be obtained with JC-1. This dye differentiates active mitochondria from inactive in the same cell because JC-1 aggregates in active mitochondria and can be seen as a red fluorescent. In inactive organelles, JC-1 has a green emission.

To date such studies have been carried on model species such as *D. reiro, X. laevis, D. melanogaster* or *M. musculus* and have mostly been concerned with oogenesis and embryo development. The obtained results showed that although there is a large amount of mitochondria in germ-line cells only a small number of those organelles remain active. This behavior is clearly different than the one observed in somatic cells.

In our studies we focused on the activity and distribution of mitochondria during spermatogenesis in the male germ-cell clusters in the earthworm *D. veneta*. We examined five stages of the development of male germ-line clusters: spermatogonia, spermatocytes, isodiametric spermatids, elongating and elongated spermatides. Mitochondria distribution was observed using the Mitotracker Red and immunofluorescent staining with anti MnSOD antibody. In order to study any changes in mitochondria activity during the development of germ-line cells cysts, JC-1 and confocal microscopy were used, which allowed us to measure the volume of the active and inactive fraction of mitochondria in the entire cell.

Symbiotic microorganisms of *Acanthococcus aceris*, *Gossyparia spuria* and *Greenisca brachypodii* (Hemiptera, Coccoidea: Eriococcidae) – ultrastructural and molecular characterization and transovarial transmission

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Symbiotic microorganisms associated with insects play a crucial role in their biology, ecology and evolution. The occurrence of symbionts in sap-feeding hemipterans is connected with their unbalanced, deficient in essential nutrients diet. Numerous investigations have indicated that symbionts synthetize the lacking substances and provide them to their host insect (Douglas, 2009).

The aim of this research was to analyze the symbiotic systems of three species of scale insects belonging to the Eriococcidae family: *Acanthococcus aceris*, *Gossyparia spuria* and *Greenisca brachypodii*. Histological and ultrastructural observations have revealed that *A. aceris* and *G. spuria* are hosts for one type of symbiotic bacteria, whereas *G. brachypodii* possesses two, morphologically different types of symbionts. Endosymbionts of *G. brachypodii* are localized in the separate bacteriocytes, whereas rod-shaped symbiotic bacteria of *A. aceris* and *G. spuria* are harbored in the cytoplasm of the fat body cells.

Molecular analyses based on 16S rDNA sequences have clearly indicated that the rod-shaped microorganisms harbored in the body of *A. aceris* and *G. spuria* are closely related to the widely distributed bacterium *Burkholderia* (Betaproteobacteria) whereas symbionts of *G. brachypodii* belong to gammaproteobacterial genera *Moranella* and *Arsenophonus*.

Endosymbionts of investigated species are characterized by the similar mode of the transovarial transmission. The microorganisms leave the fat body cells/bacteriocytes and invade ovarioles containing vitellogenic oocytes. The bacteria pass through the follicular epithelium in the neck region of the ovariole and enter the perivitelline space where they form cup-like structure (in *A. aceris* and *G. spuria*) or "symbiont ball" (in *G. brachypodii*). Next, the symbionts infest the anterior region of the oocyte.

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Zebrafish as an animal model for McArdle disease

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McArdle disease (glycogen storage disease type V) is caused by inherited mutations in the skeletal muscle-specific isoform of glycogen phosphorylase gene (PYGM). This enzyme, also called myophosphorylase, catalyses the first step of glycogenolysis. So far more than 150 different mutations of PYGM gene, which result in deficiency of this key enzyme in muscle metabolism, were found. The two most common mutations p.R50X and p.G205S cause premature termination of protein translation or disrupts the functioning of the enzyme respectively. McArdle disease symptoms include reduced ability to increased physical activity, premature fatigue, muscle pain and/or muscle crumps which sometimes leads to myoglobinuria. Currently no effective treatment is available although regular, moderate aerobic exercise can mitigate the severity of the disease (Migocka-Patrzalek et al., 2015).

Zebrafish (*Danio rerio*) as an animal model for McArdle disease is a promising perspective for further research. The zebrafish, as a model organism, present some unique advantages such as rapid production of a large number of externally developing, diploid embryos, which easily absorb chemicals. More over their transparency allows for a real-time imaging of all developmental stages. Zebrafish muscles share also many structural, histological and functional similarities with human muscles (Plantié et al., 2015).

Our goal is to obtain the zebrafish research model for McArdle disease. We show that the myophosphorylase distribution changes during normal zebrafish development from random (24hpf) to regular (72hpf) pattern in skeletal muscles. In adult fish muscles myophosphorylase locates near the Z-line. The protein expression level increases during development and this change is connected with fish movements. The addition of tricaine mesylate, the muscle relaxant that prevents growing fish from moving, cause reduction of myophosphorylase level.

The zebrafish research model for McArdle disease can be used to deepen knowledge about molecular mechanism of the disease and can be applied in screening and testing new drugs.

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Development of the male-specific SCAR marker in Japanese hop (*Humulus japonicus*)

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Humulus japonicus Siebold & Zucc. commonly known as Japanese hop, belongs to the family Cannabaceae, which is the only angiosperm family covering exclusively dioecious species with heteromorphic sex chromosomes.

H. japonicus is characterised by a multiple (polymorphic) sex chromosome system (2n=16, XX in females and 2n=17, XY1Y2 in males) which distinguishes it from two other Cannabaceae species, the common hop (*H. lupulus*) and common hemp (*Cannabis sativa*), both possessing 2n=20 chromosomes and the simple XX/XY chromosome system. According to the most common hypothesis, the polymorphic sex chromosome system derives from the simple ones by fusion between an autosome and a sex chromosome (Ohno 1967, White 1973). Grabowska-Joachimiak et al. (2011) suggested that the chromosome complement of *H. japonicus* could have arisen by the X-autosome plus autosome-autosome fusions. If so, the derived XX/XY1Y2 system of *H. japonicus* should possess an ancestral Y chromosome, present also in *H. lupulus*. The occurrence of male-specific markers amplified by the same primers in two *Humulus* species, differing in sex chromosome systems, could confirm this supposition.

From seven RAPD primers that generated Y-specific fragments in *H. japonicus*, two (OPJ-09, OPU-08) produced also Y-specific bands in *H. lupulus*. Interestingly, the amplified fragments showed different size in the compared species, and STS markers designed on the basis of OPJ-09 RAPD fragments were species-specific. It suggests that Y-specific sequences, most probably inherited from a common ancestor, underwent further differentiation in both species.

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Fertilization in angiosperms – how much different from the fertilization in animals?

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Fertilization is a key-step in sexual reproduction. Differences in the course of fertilization in angiosperms and animals are presented, with the strength point on the unity of basic mechanisms.

Majority of the sperm cells have got the motility apparatus, and only seed plants represent an unique mechanism in sperm transport – a pollen tube which delivers two sperms to the embryo sac inside of the ovule where two female cells become fertilized: the egg cell and the central cell. In majority of organisms sperms exhibit positive chemotaxis and chemokinesis to the egg cell. In flowering plants, haptotaxis and chemotropism of the pollen tube replace the sperm chemotaxis. Mammal sperms show the chemotaxis only during capacitation which is required for the acrosome reaction and egg cell penetration.

During the acrosomal reaction, the inner sperm head membrane is exposed and specific proteins are presented to the egg surface. Until now no processes corresponding to the capacitation and acrosomal reaction were found in plants. However, in angiosperms, the sperm cells are covered by the vegetative cell membrane which is rejected before sperm cell delivery to the gamete fusion site. One can't judge if that process corresponds to the acrosome reaction in animal sperms.

Interaction of the egg cell glycoproteins and sperm cell proteins is involved in gamete recognition. In flowering plants, the pollen grain or the pollen tube are recognised by the pistil tissues, and pistil glycoproteins interact with pollen / pollen tube proteins. It is not known if the angiosperm gamete recognition occurs during double fertilization.

Plasmogamy occurs after direct contact of the sperm and egg plasma membranes. Fusogenic proteins seem to be involved in plasmogamy. They form homodimers in the adjacent plasma membranes and the monomer separation leads to the phospholipid bilayer reorganisation. No such proteins were found in angiosperm gametes until now. It appears that during angiosperm gamete fusion calcium ions are involved which stimulate inverted micelle formation in neighbour membranes, and thus membrane fusion after lipid bilayer reorganisation.

The next step in fertilization is the karyogamy, and finally the zygote is formed. To avoid disturbances in zygote formation, no additional sperm can fuse with the fertilized female cell. This is ensured by the polyspermy block. The first step in polyspermy block is fast and transient, and it is related to the egg membrane depolarisation followed by repolarisation. Such a fertilization potential was observed in animals, algae and plants. Membrane depolarisation results in calcium wave in the fertilized animal egg. This is a trigger for the core reaction resulting in permanent changes in the egg envelope which block the penetration of additional sperms. In algae (e.g. *Fucus* sp.) and angiosperms (e.g. *Zea mays*), the permanent polyspermy block is provided by cell wall synthesis. Finally, the zygote starts to divide and to form an embryo – this is however a topic for another lecture.

Callose deposition in young ovules of apomictic *Chondrilla* species

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Callose, a β-1,3-linked homopolymer of glucose containing some β-1,6 branches, may be considered as a cytological marker for an early identification of the reproduction mode within the angiosperms. In the majority of sexually reproducing plants, the isolation of the spore mother cell and the tetrad by callose walls is a striking feature of both micro- and megasporogenesis. Contrary to sexual reproduction, the pattern of callose deposition is altered in the ovules of apomicts. As a rule, callose is absent in the walls of the cells that initiate diplospory and apospory, which suggests that these cells do not share identity with functional megaspore mother cells (Bicknell and Koltunow, 2004). However, a preliminary analysis of apomictic *Chondrilla juncea* ovules showed the presence of callose during diplosporous embryo sac formation (Kościńska-Pająk, 2006). Recently, callose deposition has also been documented during meiotic diplospory in an apomictic dandelion (Musiał et al., 2015). Nevertheless, research data on callose accumulation and degradation in the ovules of apomicts are limited and still remain poorly documented.

The aim of the present study was to examine the pattern of callose deposition during megaspore formation in diplosporous *Chondrilla juncea* and *Ch. brevirostris*. In both of the analyzed species, aniline blue staining revealed the pre-meiotic stages characterized by complete absence of callose within ovules. Callose appeared in the megasporocyte wall at the early prophase I and it was expressly accumulated at the micropylar pole of the cell. After a disturbed first meiotic division, a restitution nucleus formed and callose continued to be accumulated mainly in the micropylar region of the megasporocyte but a weak fluorescence of callose was also noticeable at its chalazal pole. The second meiotic division resulted in two unreduced megaspores. At this stage, callose-containing wall was distinctly visible between the diplodyad cells, whereas the side walls contained only small deposits of callose. The chalazal cell of the diplodyad developed into a functional megaspore, while the micropylar cell degenerated. A gradual degradation of callose in the wall of the functional megaspore, and simultaneously its excessive accumulation around the degenerating cell, were observed.

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RAPD technique in the genetic analysis of *Capsicum annuum* L. twins

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The polyembrony phenomenon can be observed in many species of plants and the additional embryos are created in the result of haploid or diploid embryogenesis. The polyembryony frequency and the twins number depend on a genotype and are usually low. The cytological or cytogenetical analysis of the twins origin are very laborious and the examined material is deteriorated during experimental procedures. The phenotypic and molecular evaluation of plants from a single seed may be a supplementary tool in the analysis mentioned above. In many cases the twinning is the result of the zygotic proembryo division and their genotype is identical. The new genetic variation can be expected if the additional embryos will be created in vivo as the result of haploid parthenogenesis or androgenesis with connection of the spontaneous diploidization.

Four pairs of diploid twins of *C. annuum L.*, originated from F2 seed and their F3 progeny were the subject of investigation. The F1 hybrid has been obtained by the cross-fertilization of red-fruited ATZ breeding line with yellow-fruited cultivar `Sono`. The ripe fruit of F1 hybrid were red. Two pairs of twins (1A *vs*. 1B) and (4A *vs*. 4B) have produced red fruit. The main fruit weight of plants within pairs was 55 g *vs*. 85 g and 126 g vs. 39 g respectively. The plants of pair (3A *vs*. 3B) set yellow fruit. One plant of the last pair gave yellow fruit (2A) while from the second plant (2B) the red fruit were picked. In the RAPD analysis 26 primers, presented below, were used: A02, A04, A05, A06, A07, A08, A09, A10, A12, A18, A19, A20, 1A,OPA03, OPAE11, OPAF07, OPAG01, A11(2), 3A(3), F06(3), OPAE10(2), OPAF04(3), OPB10(9), OPE19(1), OPF05(3), OPP09(6). Seventeen primers have generated the monomorphic products only. The number of the polymorphic products is given in the parenthesis beside the symbols of the starters.

The biggest genetic differences (coefficient of genetic distant – 0.174) has been observed between the diploid twins marked 1A *vs*. 1B. Lower variability (0.138) were noted for twins differed with regard to the fruit colour. For the pair 4A *vs*. 4B the genetic distant coefficient was noted as 0.048. No any RAPD polymorphic product has been obtained for the plants within pair 3A *vs*. 3B. The coefficient of genetic distant between these twins and F1 hybrid reached 0.038. For the other twins vs. hybrid mother plant the coefficient ranged from 0.018 to 0.179. On the basis of the data presented above the origin of twins 3A and 3B may be explained as the division of the zygotic proembryo. For other twins the development of embryos from recombined haploid cells (parthenogenesis and/or androgenesis) with spontaneous chromosome doubling should be pointed. The progeny of twins, excluding pair 3A *vs*. 3B were uniform in their phenotype within F3 population. The RAPD technique seems to be good tool in the genetic analysis of additional embryos.

Global analysis of cell adhesion genes in sexually differentiating gonads of mouse

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Mouse testis and ovary structure forms upon sexual differentiation of gonads. In this process a mass of unorganized cells starts to sort and aggregate into compartments: testis cords vs. interstitium in the XY gonads, or germ cell nests vs. stroma in XX gonads. Specifically regulated expression of cell adhesion molecules (CAMs) at the cell surface is thought to be decisive for sorting of cells. We aimed to investigate the expression of all CAMs and factors regulating cell adhesion during gonadal development in mouse fetuses at 11.5, 12.5, and 13.5 *dpc* (days *post coitum*). To investigate which CAMs are specifically expressed in supporting, interstitial/stromal and germ cells in XY and XX gonads we used transgenic mouse lines expressing *EGFP* reporter gene under control of four promoters of genes expressed in three cell lines: *Sry* and *Sox9* for supporting cells (pre-Sertoli and pre-granulosa cells), *Mafb* for interstitial/stromal cells, and *Oct4* for germ cells. Gonadal cells were sorted using FACS. mRNAs were analyzed using microarray supported by qPCR. We detected expression of 129 genes involved in cell adhesion. Although a great number of CAMs expressed at even level in all three studied cell lines, we identified a series of differentially expressed cell adhesion genes. Cadherin-5, -6, -10, protocadherin-β17, -β19, -β20, NCAM1 and integrin-α8 were upregulated both in XY and XX interstitial/stromal cells, whereas VCAM1, Notch3 were upregulated specifically in XY interstitial cells. A striking similarity in CAMs expression was characteristic for XY and XX germ cells; high expression of claudin-7 and -20, cadherin-1, protocadherin-9, PECAM-1 and integrin-β3 was detected in this cell line. We also showed a strong diversity in expression of CAMs in XY and XX supporting cells. Claudin-6 and -11, cadherin-9 and -23, nectin-1 and integrin-α3 were specifically upregulated in pre-Sertoli cells, whereas cadherin-9 was upregulated in pre-granulosa cells. Significant upregulation of many CAMs was characteristic for XY supporting cells, whereas in XX equivalents many CAMs were downregulated and only few were upregulated. Such regulation of CAMs expression may be responsible for creation of testicular or ovarian structure. A potential increase in cell adhesion in differentiating XY gonads may lead to distinct aggregation of supporting cells into solid testis cords, whereas in XX differentiating gonads pre-granulosa cells only loosely surround nests of female germline cells. Global analysis of CAM expression allows to clarify mechanisms of testis and ovary structure development and can contribute to understanding of the origin of their cell lineages.

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The fate of integumentary cells in apomictic and sexual members of Asteraceae (genera: *Taraxacum*, *Hieracium* and *Pilosella*)

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Some members of the Asteraceae family have specific pathway of cell differentiation in the integument. The specific and specialized integument tissue is called nutritive tissue or periendothelial tissue (e.g. Musiał et al., 2013; Kolczyk et al., 2014). We studied ultrastructure of this tissue in the apomicts and also sexual species and obtained similar results in both types of plants. In members of all three examined genera (*Taraxacum*, *Hieracium* and *Pilosella*) integument periendothelial zone consisted of mucilage cells. Mucilage was deposited as a layer between the plasma membrane and the cell wall. In mucilage integument cells the plasmodesmata become elongated and associated with cytoplasmic bridges in *Taraxacum* (Płachno et al., 2015) as well in other examined genera. Finally protoplasts had an irregular shape and were degenerated. After cell-wall breakdown of mucilage cells the cavities filled with mucilage were formed.

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Cultured endosperm versus endosperm in bread wheat caryopsis: biochemical, ultrastructural and molecular studies

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Cereals plays crucial role as a food source for human population and an animal livestock. Consumers are especially interested in grains with endosperm as a storage tissue accumulating carbohydrates and proteins. In our previous research we have revealed (Popielarska-Konieczna et al., 2013) that the immature endosperm of bread wheat (*Triticum aestivum*) isolated at 7–8 days post anthesis can be maintained under in vitro conditions. Histological studies showed that explants could be divided in active and inactive part of tissue. The actively growing part of the explants accumulated starch granules, while in non-growing tissue no starch grain accumulation was detected. Ultrastructural studies showed characteristic ribbed cell walls and typical starch grains, which can be classified as A and B type. The observation of FDA stained tissue suggested that active tissue is viable during at least four weeks of the culture. The analysis of internucleosomal fragmentation of DNA suggested the induction of apoptosis in cultured endosperm affects only inactive part of the endosperm. DNA degradation in active part of explant was rather typical for necrosis. The content of starch detected in cultured explants was significantly higher on medium with 10% of sucrose in contrast to medium with 3% of sucrose. Additionally the amount of starch was similar to level of *in planta* maintained caryopsis at the same age and held until four weeks of the culture. Decreasing of starch content was observed after 10–14 days of culture on media with 3% sucrose. Explants subcultured on media with 3% sucrose and abscisic acid (ABA) showed threefold higher amount of starch than explants on media without ABA. Analysis of storage proteins carried out by polyacrylamide gel electrophoresis (SDS-PAGE) showed that glutenins were present only in active tissue and with lower amount than in caryopsis at the same age. On the other hand RNAseq analyses revealed a different composition of storage protein gene transcipts compared to the composition found in caryopsis. Our results suggest that manipulation of storage materials accumulation in isolated cultured endosperm of cereals is possible.

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Prenatal development of the oviduct in cat (*Felis silvestris catus*)

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In the prenatal development of the oviduct in cat can be determined two stages. The first undifferentiated stage lasts from about 25th day p.c. to 42nd day p.c. and involves formation and growth of the paramesonephric ducts. The second stage lasts from 44th day p.c. to 63rd day p.c. and involves the histogenesis of the wall of oviducts.

The studies were carried out on the female cat fetuses, aged from 25th day p.c. to 63rd day p.c. The methods used in the studies were: the light microscope (LM) observations of the histological serial slides of oviducts and the scanning electron microscope (SEM) observations of the microcorrosion vascular casts of oviducts.

The research revealed that in the first stage the oviducts are the cranial segments of the paramesonephric (Müllerian) duct. These ducts are composed of the homogenous mesenchymal tissue, that is lined by the pseudostratified epithelium. The pseudostratified epithelium has the same height over the entire cross-section of the ducts and consist of the cylindrical cells, spindle-shaped cells, club-shaped cells and pyramidal cells. At this stage of development the paramesonephric ducts co-localise with the wider mesonephric (Wolffian) lined by the simple cuboidal epithelium. On the 36th day p.c. the cranial parts of the paramesonephric ducts is supplied by single longitudinal blood vessel, that gives the small branches creating the simple outer vascular network with single mucosal capillaries. On corrosion vascular cast many places of intussusceptive angiogenesis are visible. Between the 41st–43rd day p.c. the oviduct's main blood vessels mature and became the arterioles and venules running on oviduct wall as vascular pairs.

In the second stage the cranial parts of paramesonephric ducts apparently differentiate structurally into the oviducts. The wall of the oviducts is consists of the partly pseudostratified epithelium and thick connective tissue lamina propria mucosae and thin muscular layer. The reduced in size mesonephric ducts stay still laterally in wall of oviduct. At the end of the prenatal period the wall of oviduct posses well-developed vascular system, which consists of the well developed subserosal and mucosal vascular networks.

Limb girdle muscle dystrophies: genetic, clinical, and biochemical diversity in Polish patients

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Myopathies are clinical disorders of the skeletal muscles, in which the muscle fibers do not function properly, resulting in muscular weakness. Based on that whether they are inheritable or not myopathies could be classified as (*i*) acquired myopathies including inflammatory myopathy, infection (myositis), toxic myopathy and myopathy associated with systemic diseases, and (*ii*) inherited myopathies including congenital myopathies, metabolic myopathies, mitochondrial myopathy and muscle dystrophies.

Muscular dystrophies form a large group of myopathies that are highly variable, both phenotypically and genetically. Among them are limb-girdle muscular dystrophies (LGMDs), showing progressive limb girdle weakness (sparing the facial and distal muscles) with autosomal patterns of inheritance. Studies performed world-wide revealed that so far mutations in 31 muscle-specific genes were found to be associated with the disease, and mutation(s) within each gene was associated with the particular phenotypic LGMD subtype. Molecular pathophysiology of the LGMDs is heterogeneous: mechanisms of the disease range from defects involving in proteins of dystrophin-dystroglycan complex, defects of muscle membranes, enzymatic defects, sarcomeric and nuclear lamina dysfunctions. Moreover, it has been shown in numerous studies that mutations within the same gene are able to cause variable symptoms hindering diagnosis.

Whole exome sequncing was applied to identification of causative mutations in Polish LGMD patients. So far we found mutations in several genes, including *DYSF* and *ANO5* genes. *DYSF* encodes dysferlin, a transmembrane protein that has important role in skeletal muscle repair, and *ANO5* encodes anoctamin-5, a putative calcium-activated chloride channel. Mutations in these genes are known to cause of LGMD 2B and LGMD 2L types, respectively. Several point mutations in *DYSF* in 7 patients, and two mutations in *ANO5* in one patient have been identified, and some of them are novel. The muscle biopsies of those patients were subjected to western blot and immunohistochemistry analyses.

Both genetic, morphological and biochemical analysis revealed that LGMDs could be in fact oligogenic and not monogenic diseases.

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The influence of the environmental endocrine disrupting compounds (EDCs) 17α-ethinyl estradiol and bisphenol A on the development of gonads in *Xenopus laevis*, *Bufo viridis* and *Hyla arborea*

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Natural and synthetic endocrine disrupting compounds (EDCs) are increasingly detected in the aquatic environment. These substances interfere with the endocrine system and can affect the sexual development of vertebrates, including amphibians. We tested the influence of the artificial estrogen 17α-ethinylestradiol (EE2) and the plasticizer bisphenol A (BPA) on differentiation and development of male and female gonads in three deeply divergent anurans: the model-species *Xenopus laevis* (Pipidae) and two non-models, *Hyla arborea* (Hylidae) and *Bufo viridis* (Bufonidae). Although the impact of EE2 and BPA on the somatic amphibian ontogenesis has been investigated, knowledge about effects on the sexual development in non-model anurans remains poor. Our new approach combined simultaneous exposure of tadpoles to three concentrations of EE2 (50, 500, 5,000 ng/L) and BPA (0.023, 2.28 and 228 μg/L) in a flow-through-system (Tamschick et al. 2016 and in prep.).

Our study applies genetic sexing of metamorphs followed by histological examination to detect potential feminizing or masculinizing effects and possible impairments of gonad development and differentiation. We found striking species-specific differences in the vulnerability to EDCs. We detected gonadal impairments as sex reversal, mixed sex, partial or total sterility, and gonad underdevelopment or shortening. EE2 caused mainly genetic-male-to-phenotypic-female sex reversals and mixed sex gonads, whereas BPA was less harmful and caused mainly shortening of gonads and retained segmentation (metamery) of testes.

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The structural and ultrastructural comparative studies of thyroid differentiation in grass snake (*Natrix natrix* L.) and sand lizard (*Lacerta agilis* L.)*

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The thyroid gland has a long evolutionary history and it is the first endocrine structure to become recognizable during an animal's development. Even though the thyroid gland is structurally conserved in all vertebrate species, exhibiting a similar follicular structure and function, there are some gross morphological differences among species, and the responses of this structure to environmental influences are also different across the phylum. The thyroid gland consists of individual structural and functional units-follicles that evolutionarily descended from the ancestral endostyle of primitive Chordata. The purpose of this study was to compare developmental process of thyroid gland differentiation in grass snake *Natrix natrix* L. and sand lizard *Lacerta agilis* L. The eggs of the *Natrix* and *Lacerta* were incubated in the constant temperature at 30°C and the embryos were isolated, starting at eggs lying and finishing at hatching of the first individuals. The age of *Natrix* embryos was calculated using the table of species development (Rupik, 2002) but the age of *Lacerta* embryos was calculated using the developmental table of Peter (1904). The ultrastructural findings show that: during early developmental stages, cells of the thyroid primordium in sand lizard embryos had ultrastructural features that were basically similar to those of the thyroid primordium in grass snake. However, single cells that contain giant lipid droplets have been found within the undifferentiated cellular cords in embryos of *Lacerta*. Similar cells were not found in early thyroid primordium of grass snake. Even though the follicular lumen in sand lizard embryos is differentiated by cavitation similar to that in the grass snake, there were very important differences during the early stages of the differentiation of the cellular cords and the formation of the thyroid follicles. The activity of the embryonic thyroid gland in *Natrix* and *Lacerta* are different. In grass snake embryos the activity of thyroid gradually increased, and at the time of hatching, it exhibited the features of a fully active gland. In sand lizard embryos the thyroid exhibited the features activity very early but just before hatching the activity of the gland gradually decreased.

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Developmental changes of the ultrastructure of the lingual nail in domestic duck (*Anas platyrhynchos f. domestica*) during embryonic period

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The ventral surface of the apex of the tongue in the domestic duck is covered with the orthokeratinized epithelium. This epithelium is compose of the basal, lower and upper zone of intermediate layer and keratinized layer. The keratinized layer of the orthokeratinized epithelium forms specific structure named lingual nail. The lingual nail in the domestic duck extend to the front and sides of rostral part of the tongue and is the external skeleton of the apex. Functionally the lingual nail act as spoon to collect grains during pecking.

The aim of the present study was to characterize ultrastructure of the particular layers of the orthokeratinized epithelium with special attention to the structure of the lingual nail in adult domestic duck and also determine the ultrastructural changes in the epithelium during embryonic period. The results will help to evaluate if the structure of the orthokeratinized epithelium and its keratinized layer – lingual nail is fully development before hatching and prepare to fulfill function during pecking.

TEM observations of the orthokeratinized epithelium in adult show a gradual reduction in cell organelles in the following epithelial layers, at increasing amounts of keratin fibers. Keratin fibers in the lower zone of the intermediate layer are arranged in bundles interspersed in different directions. In the upper zone are loosely arranged in bundles along the cell nuclei. A characteristic feature of the lingual nail is that it is composed of two types of cells, i.e. electron light and dark cells. The electron dark cells are tightly filled with long, horizontally oriented bundles of keratin fibers, while in more superficial, electron light cells, the keratin fibers are short and loosely arranged. The cell membranes in the lingual nail, even on its surface, are mutually invaginate and connected by desmosomes.

The orthokeratinized epithelium on the ventral surface of the apex develop during three stages: embryonic stage, transformation stage and prehatching stage. In the embryonic stage epithelium is built of 3–4 cell layers, which are characterized by similar cell shape and had similar ultrastructure. The small intercellular spaces are observed between cells and cell membrane are connected by desmosomes. In cell cytoplasm are present mitochondria, rough ER, ribosomes and glycogen. During transformation stage, which lasts from 14^{th} till 20^{th} day, the epithelium is built of three layer: basal, intermediate and superficial layer. Additionally, due to the shape of cells and their nuclei, in the intermediate layer are seen two zones, lower and upper one. The keratin fibers are seen in the intermediate and superficial layer and cell membrane are connected with numerous of desmosomes. During the prehatching stage, which lasts from 21^{st} till 25^{th} day, the keratinized layer – lingual nail is formed and is covered with the superficial layer. The characteristic features is presence of specific, periderm granules in the superficial layer. About $24/25^{th}$ day the superficial layer is absent.

The results of the ultrastructural observation showed that orthokeratinized epithelium and the lingual nail is fully developed before hatching and ready to perform their function during pecking.

Low seed set and its improvement induced by biostimulants in self-incompatible cultivars of buckwheat (*Fagopyrum esculentum*) with disturbed proportion of Pin:Thrum flowers

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The buckwheat (*Fagopyrum esculentum* Moench), economically important crop, is a distilic, self-incompatible plant producing morphologically different Pin (long styles, low anthers) and Thrum (short styles, high anthers) flowers (Cawoy et al., 2009). The aim of the research was: 1) to determine the stages of embryological development that negatively influence the effectiveness of generative reproduction of four cultivars (Kora, Panda, PA13, PA14) with low (8–52%) seed set, and 2) to improve their productivity by increasing seed set and/or seed weight, using exogenously applied (in the beginning and in the full flowering phase) plant growth biostimulants (cysteine, NAA, GA3, Tytanit, NaCl, putrescine, BAP, ASAHI SL).

In non-treated with biostimulants plants the male line was not disturbed resulting in production of highly viable pollen estimated by Alexander dyes, contrary to the female line with degeneration of mature female gametophyte cells (premature degeneration of both synergids or degeneration of whole egg apparatus). The influence on low seed set had also embryo degenerations.

Strongly disturbed proportion of Pin:Thrum flowers in all cultivars (1:4–1:20) though negatively correlated with the presence of the compatible pollen on the stigma was not correlated with seed set. Similarly, seed set was not correlated with the frequency of legitimate crossing estimated by pollen tube germination and its length in aniline blue stained pistils.

The influence of biostimulants on seed set and seed weight was remarkable in PA14 and minor in PA13, Panda and Kora. In PA14 six to eight (dependently on the time of plants spraying) out of eight used biostimulants had positive effects resulted from increasing frequency of embryos without signs of degenerations as compared to the non-treated with biostimulants plants.

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Comparative study of 3D development of crustaceans larvae (freshwater shrimp *Neocaridina hetaeropoda*)

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Arthropods are the most diverse and common animals that live all over the world. Crustacea, which belong to that group, show an incredible diversity of morphology, size, and habitats attesting their evolutionary success. Although, they are the most abundant animals which mainly live in aquatic ecosystems, the knowledge about their morphology and development is rather fragmentary and there is still a lot of information which needs to be clarified. Moreover, most studies of anatomy and morphology of decapods are based on classical methods. The lack of general information on development and histology of crustacean digestive tract encouraged us to start this study.

A comparison of novel vs classical histological and embriological techniques employing high resolution microscopy and micro-CT scanning for 3D visualizations were used in order to present the structure and ultrastructure of digestive system of freshwater shrimp *Neocaridina heteropoda* (Crustacea, Malacostraca, Decapoda) which can serve as great example for comparative study for other crustaceans. *Neocaridina heteropoda* (Crustacea, Malacostraca) is the the freshwater shrimp that is easy to possess from the local breeders, easy to rear in the laboratory.

The structural and ultrastructural comparative studies of skin differentiation in two lepidosaurian species – sand lizard (*Lacerta agilis* L.)* and Egiptian cobra (*Naja haje* L.)**

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All lepidosaurian species are terrestrial animals that have become completely independent of the aquatic environment and therefore the skin of lizards and snakes are dry and has low permeability to water. This kind of integument protects the organism from mechanical damage and dehydration. Epidermis of adult lizards and snakes consists of six layers, built from different types of cells. It changes throughout life and periodically regenerates and is shed. The process occurs either continuously along with its exfoliation (lizards) or the epidermis is periodically renewed over the whole body in the process known as skin shedding or moulting (snakes). The functions of the epidermis and its appendages in reptilian species similarly as in other vertebrates are established during embryogenesis and they are the results of a complex and precisely coordinated stratification program. The knowledge of the process of skin differentiation in squamate species in comparison of the process of skin morphogenesis in other vertebrates is poor. The purpose of this study was to compare developmental process of skin differentiation in sand lizard *Lacerta agilis* L and Egyptian cobra (*Naja haje*). The eggs of the *Lacerta* and Naja were incubated in the constant temperature at 30°C and the embryos were isolated, starting at eggs lying and finishing at hatching of the first individuals. The age of *Lacerta* embryos was calculated using the developmental table of Peter (1904) and the age of *Naja* was calculated using the table of species development (Khannoon and Evans, 2014). Based on structural and ultrastructural investigation, the embryonic development of the sand lizard and Egiptian cobra integument was divided into four phases. Our studies show that: during early developmental stages, cells was made of layers in embryonic epithelium of both studied species were basically similar. The differences between structure and ultrastucture of differentiated skin in *Lacerta* and *Naja* appeared just at the end of embryos development. The shedding complex in both compared reptilian species is different that is a reflection of adaptation to different environmental conditions. Moreover, the most outer layer of developing epidermis – periderm in *Lacerta* consists only of one cellular layer but in Egiptian cobra is made of two layers.

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**All specimens used in the experiment were captured according to the Egyptian regulations concerning the protection of wild species (Convention on Biological Diversity ratified in 1992 and 1994). The Egyptian cobra is not included in the Washington Convention of 1973.

Modes of transovarial transmission of symbiotic microorganisms in Hemiptera: Sternorrhyncha

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Sternorrhynchans (aphids, scale insects, psyllids, whiteflies) are widely distributed pests which feed on plant sap. Since the diet of these insects is deficient in amino acids, they live in mutualistic associations with microorganisms (bacteria or yeast-like symbionts) which synthesize substances missing in the diet. The symbionts are harbored in the cytoplasm of giant cells termed bacteriocytes (containing bacteria) or mycetocytes (containing yeast-like symbionts). Symbiotic microorganisms are transmitted from the mother to the offspring transovarially (i.e. via female germ cells). Results of numerous studies show that sternorrhynchans developed variable modes of ovary infection by symbionts, however, the beginning of the symbiont transmission (i.e. release of bacteria from the bacteriocyte cytoplasm into the body cavity) is correlated with the development of the germ cells. In viviparous aphids symbiotic microorganisms invade the posterior pole of the embryo at the blastula stage. In oviparous aphids the bacteria pass through the follicular epithelium surrounding the posterior pole of the terminal oocyte at the stage of choriogenesis. The bacteria may migrate through the cytoplasm of follicular cells or through the spaces between neighboring cells. Next, the symbionts via the perivitelline space enter the oocyte cytoplasm. In scale insects, symbionts may infest undifferentiated germ cells (cystocytes) in larval ovaries (e.g. in *Marchalina hellenica, Puto albicans*) or oocytes at the stage of vitellogenesis or choriogenesis in the ovaries of adult females (in most scale insects). The bacteria may invade follicular cells surrounding the anterior pole of the oocyte (in Pseudococcidae, Eriococcidae) or its posterior pole (in most scale insects). In contrast to the situation in aphids, in scale insects the symbionts do not enter the oocyte cytoplasm, but accumulate in the perivitelline space. In psyllids, the bacteria invade the ovarioles containing choriogenic oocytes. The symbionts pass through the cytoplasm of follicular cells and enter the perivitelline space where they gather forming a characteristic symbiont ball. In whiteflies, in contrast to other sternorrhynchans, whole intact bacteriocytes migrate into the ovaries containing vitellogenic oocytes. The bacteriocytes enter the perivitelline space through the spaces between neighboring follicular cells surrounding the posterior pole of the oocyte and gather in the invagination of the oolemma.

In vitro propagation and photosynthetic apparatus development in energy hybrid sorrel

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Rumex tianschanicus × *Rumex patientia* is a cross between English spinach (*R. patientia* L.) as the female line and Tien Shan sorrel (*R. tianschanicus* A. Los.) as the male line. The hybrid sorrel is suitable for fuel biomass cultivation as a renewable source of energy, can be used for biogas, pellets, briquettes production and may be of interest also as an important medicinal plant. We developed an efficient and reproducible micropropagation protocol for hybrid sorrel through direct shoot bud induction from hypocotyls (Ślesak et al., 2014). The highest frequency of organogenetic response was obtained on MS medium supplemented with 0.17 mg/l IAA, 2.2 mg/l BAP and 2% sucrose. Histological and SEM analyses confirmed direct organogenesis and showed a membranous-fibrillar structure, which was similar to the extracellular matrix (ECM), around non-morphogenic callus cells. The amount of nuclear DNA in leaves of plantlets regenerated in vitro, estimated using flow cytometry, was similar to donor plants (about 4.6 pg/2C), which confirmed that developed protocol assures the stability of nuclear DNA content. This system could be useful for rapid clonal propagation and genetic transformation. Our present experiments focused on the photosynthetic apparatus development during in vitro culture. The lowest photosystem II operating efficiency (ΦPSII) was observed for unrooted adventitious shoots, regenerated in vitro from explants. ΦPSII values, showing real photochemical efficiency of PSII in light, were similar for rooted regenerants in both in vitro and ex vitro conditions. Moreover, the results for linear electron transport rate in PSII (ETR II), and non-photochemical quenching (NPQ), which is a measure of excess energy dissipation as heat, were also similar as data for ΦPSII. The obtained results strongly indicated that rooting of explants under in vitro conditions play a key role in photosynthetic apparatus efficiency rather than their adaptation to ex vitro conditions. The correlation between chloroplasts ultrastructure and photosynthetic activity in the following stages of in vitro culture: 1) regenerated adventitious shoots, 2) the same shoots after in vitro rooting and 3) after acclimatization to *ex vitro* conditions was studied using TEM analysis.

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Ovary structure and oogenesis in a basal clitellate annelid *Capilloventer australis* (Capilloventridae)

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Capilloventridae is a small family of both marine and freshwater clitellate annelids. Some morphological characters (as the presence of hair as well as bifid chaetae in all somatic bundles located dorsally and ventrally) and molecular studies suggest that Capilloventridae is the putative sister taxon to all other Clitellata. However, the biology and anatomy of these tiny annelids is still poorly known. The aim of the presented studies was to analyze the ovary organization and oogenesis in a capilloventrid representative from Australia.

Recent analysis (Świątek et al., 2016) have revealed that the paired ovaries of *Capilloventer australis* are located in segment XIII. Each ovary has a form of long chain composed of linearly arranged growing germ cells covered by a thin envelope made up of somatic cells. The ovary tip, connected via a thin ligament to the intersegmental septum, is occupied by oogonia followed by previtellogenic and early vitellogenic oocytes. Filled with yolk spheres vitellogenic oocytes detach from the ovary and float freely in the segment lumen. Vitellogenic oocytes are no longer enveloped by somatic cells. The course of oogenesis found in *C. australis* is similar to that found in other Clitellata with one important exception – in contrast to the other clitellate annelids, the germ cells in *C. australis* ovaries develop individually and do not from syncytial cysts. Serial sectioning on ultra-thin sections did not revealed the presence of intercellular bridges between germ cells. This result is surprising because in all clitellate annelids studied so far both male and female germ cells form cysts equipped with central cytoplasmic mass, the cytophore.

At present it is not clear whether the lack of germ cysts is a basal or derived condition for Capilloventridae.

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Female reproductive system morphology in earwigs and their reproduction strategies

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The Dermaptera is a small insect order with around 2200 species that are traditionally classified into 9 families. The representatives of three families (Spongiphoridae, Chelisochidaea and Forficulidae) are grouped together and form the so-called advanced earwigs, the Eudermaptera. Among earwigs three reproductive strategies have been described: ovoparity, viviparity and ovoviviparity. Comparative analyses of the female reproductive system have led to the following conclusions: (1) there are significant differences in the morphology of the female reproductive system between representatives of basal and advanced earwigs; (2) the morphology of the ovaries of viviparous *Arixenia* shows several similarities to the ovaries in advanced earwigs; (3) there are specific modifications in the morphology of the female reproductive system and the course of oogenesis in *Arixenia* that are related to the viviparity of this species; (4) there are not characteristic modifications in the morphology of the female reproductive system in ovoviviparous species related to that reproductive strategy.

Organization of the female germ-line cysts in the potworm *Enchytraeus albidus* (Annelida: Clitellata)

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These studies are dedicated to Professor Janusz Kubrakiewicz who was involved in studies on gametogenesis and germ-line cyst formation in *Enchytraeus albidus.*

Enchytraeids (potworms) are cosmopolitan representatives of mesofauna. Like earthworms, they play an important role in the decomposition of organic matter; however, some aspects of their biology are still not known or at the least are unclear. One of these fields involves the structure of germ-cell cysts (clusters) within the ovaries.

In *Enchytraeus albidus*, the paired ovaries are bush-like and are composed of numerous, clearly separated germ-line cysts that consist of germ cells in subsequent stages of oogenesis. The ovaries are polarized (a proximal and a distal part can be distinguished) and germ-line cysts in the consecutive stages of oogenesis are localized along the long ovary axis. During the advanced stages of oogenesis, late vitellogenic oocytes detach from the ovary and flow freely in the coelomic fluid.

The female germ-line cysts of *E. albidus* have the same organization as the other clitellate annelids known to date. The entire cyst forms a kind of syncytium in which the germ cells are located at the periphery of the cyst, whereas the cyst centre is occupied by a central and common cytoplasmic mass (cytophore). Each germ cell is connected to the cytophore via one stable cytoplasmic bridge (ring canal). The germ-line cysts in *E. albidus* are composed of 16 germ cells and this is the lowest number of germ cells within a single cyst that has been described in clitellate annelids to date. During oogenesis, the fates of germ cells within a given cyst differentiate into two categories – one oocyte and15 nurse cells.

Our study also revealed rich cytoskeletal components within the germ-line cysts. Both actin filaments and microtubules were observed. The actin filaments primarily formed an inner rim of ring canals that probably stabilizes these open channels during oogenesis. The microtubules form a network in the cytoplasm of the germ cells and cytophore but their role is as yet unknown.

In conclusion, the simple 16-cell cysts in *E. albidus* appear to be a good model to study the formation and functioning of female germ-line cysts in clitellate annelids.

Myosin VI in mouse spermatogenesis

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Myosin VI (MVI) is an unconventional motor protein which moves toward the minus end of microfilaments unlike all other known myosins, which are the plus end oriented motors. MVI has three domains: an N-terminal motor domain (head) with ATP- and actin-binding motifs; a neck region (lever arm) with two light chain binding sites, important for reverse direction movement; and a multi-domain tail playing role in stepping and binding cargoes and/or adaptor proteins. Due to the presence of two different inserts in the C-terminal part of the tail, small (SI) and large (LI), four alternatively spliced MVI variants are formed in mammalian cells: isoforms with SI or LI and protein variants with both or without inserts. It has been postulated that presence or lack of these inserts could determine localization and function of MVI in different cell types (Tomatis VM et al., 2013).

MVI has been implicated in a number of cellular processes, ia. endocytosis, stabilization of Golgi apparatus, exocytosis, morphology of hair cells in Corti organ, nuclear processes, autophagy, and miogenesis (Zakrzewski and Lenartowska, 2014). It was also showed that lack of MVI in *Drosophila* testes leads to male infertility, what proves that MVI function is crucial in spermatogenesis in invertebrates (Noguchi et al., 2006). However, it is still unknown whether MVI plays an important role in sperms formation or maturation in mammals. Using western blotting and RT-PCR we have showed the level of MVI expression in mouse testes in comparison to other organs. Moreover, we have revealed expressed variants of MVI during spermatogenesis. Our studies have been supplemented by ultrastructural and immunocytochemical analysis, that showed MVI's localization in sperm acrosome, acroplaxome and manchette. All obtained results reported here suggest that MVI may play an important role in spermatogenesis of mammals.

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Selected adaptive pollen characters in karyologically highly diverse pansies (Viola, sect. Melanium, Violaceae)

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The tetraploid (4x) western-Eurasian *Melanium* Ging. section of the *Viola* L. genus with ~125 species (pansies) and chromosome numbers ranged from n=2 to ~64 (Marcussen et al., 2015) occupying different habitats and elevations is a good model to study adaptive pollen traits – pollen viability and number of pollen grain apertures. 74% of pansies are pollen-heteromorphic producing 3–6-aperturate pollen grains in one flower of the individual plant (Nadot et al., 2000). The adaptive role of pollen heteromorphism in the genus *Viola* was postulated and the number of apertures was correlated with pollen longevity, pollen germination timing, environment (Dajoz, 1999) and also with ploidy level (Nadot et al., 2000). The higher number of apertures the faster pollen tube germination, but shorter ability to germinate (Dajoz, 1999). There is a correlation between pollen heteromorphism and elevation – the higher elevation the lower mean aperture number. In pansies pollen heteromorphism is not correlated with the ploidy as in other sections of the genus *Viola* (Nadot et al., 2000).

The aim of our study was to examine the correlation between pollen heteromorphism, pollen viability and the influence of environment (elevation, metalliferous soils) in pansies collected from several European countries. Pollen, stained with Alexander dyes, was analyzed in 25 taxa from ~50 populations, including metal-tolerant and occurring at different altitudes up to alpine level species. There was a positive correlation between pollen heteromorphism and viability – the higher viability the higher mean aperture number. The influence of high altitude on mean aperture numbers was not confirmed but this condition significantly affected pollen viability. In obligatory calamine (Zn/Pb) and serpentine (Ni/Cr/Fe/Mg) metallophytes, pollen heteromorphism and viability were not affected by heavy metals but in facultative metallophyte (*Viola tricolor*) 3-aperturate pollen occurred only at metalliferous populations but not at non-metalliferous ones.

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Stages of pancreas development in grass snake *Natrix natrix* L.* (Lepidosauria, Serpentes)

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Pancreas is a very important organ existing in all vertebrates species. This organ contains two distinct populations of cells, exocrine and endocrine. Exocrine cells release digestive enzymes into the gut and endocrine cells secrete hormones into the blood. It causes that the pancreas connects two glands in one what makes that studies on it are far more interesting. The development of this organ in reptilian species has been poorly known. For this reason the aim of this research was to investigate the embryonic development of the pancreas in the grass snake embryos. The studies was performed from the time of the egg laying until the hatching using standard methods for light and electron microscopy. In addition to this, 3D reconstructions of the pancreas and surrounded organs were prepared on basis of serially histological sections at different developmental stages. The pancreas anlage in the grass snake embryos first appears at the developmental stage I as two unconnected buds, dorsal and ventral. During the next developmental stages these two buds start to join together and at the developmental stage VIII are fully fused. The first endocrine cells appear at the developmental stage V at the dorsal part of the pancreas which is closely apposed to the spleen. These cells form structures resembling nonlinear cords or are located single between acini or in the wall of the acini and ducts. Ultrastructural studies have revealed that there are four major types of endocrine cells in the embryonic pancreas of grass snake. There are no evidences for existing endocrine cells in the ventral part of the pancreas. The endocrine cells are distinguished by size, shape and electron-density of their granules. At the time of the hatching the pancreas is located below the gall bladder and the spleen. The dorsal end of the pancreas penetrate the ventral end of the spleen. On basis of these results, it can be assumed that three from four endocrine cells existing in the pancreas of the grass snake correspond with the A-, B- and D-cells in adult species of this snake.

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