The variability of the atrium structure in leeches of the family Erpobdellidae Blanchard, 1894 (Hirudinida)

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Systematics of the erpobdellid leeches is still the subject of numerous discussions because of the lack of a uniform classification. This concerns both genera and species as well as higher taxonomic levels. The structure of the reproductive system should be considered as a set of valuable taxonomic characters in determining the leeches of the family Erpobdellidae (Lukin, 1976). There are a few studies concerning the description of the structure and variability observed in the reproductive system of erpobdellids (Agapow, Bielecki 1992). The structure of an atrium in this leeches is not commonly described as well (Sandner, 1951). The aim of our study was to analyze the variability of the atrium structure in order to find phenetic and cladistic relationships between the species. The analyses included 8 representatives at species level and one subspecies form belonging to the family Erpobdellidae. Leeches were collected from various reservoirs in Poland and from the Caspian Sea. The samples were preserved according to standardized procedures (Pawłowski, 1936). Dissected atria of 350 leeches were measured using a stereoscopic microscope with an accuracy of 0.1 mm. The model of atrium is described by 16 indices. The results were used to perform phenetic and cladistic analyzes. The obtained phenogram showed the species grouped in two polytypic clusters. The most similar atria were observed in *Erpobdella octoculata* and *E. nigricollis* as well as *Dina lineata* and *E. testacea*. The most parsimonious tree obtained from cladistic analysis grouped considered erpobdellids in two distinct branches: first containing *D. stschegolewi* and the second consisted of other leeches. The outcomes of our analyses appeared to be coherent with the current classification of erpobdellids based on molecular data (Siddall, 2002) where representatives of the genera *Erpobdella, Dina, Trocheta* do not form monophyletic groups. We believe that it would be valuable to include the atrial features to the set of morphological characters and perform more complex phylogenetic inference of Erpobdellidae in order to establish uniform classification of the leech family.

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Phylogeny of oligochaetes *sensu lato* (Annelida: Oligochaeta) based on characters of the female reproductive system

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Since Siddall et al. (2001) suggested to synonymize Oligochaeta with Clitellata there have been numerous discussions on systematics and classification of annelids. Many different characters, including morphological and molecular data, were involved to find the most reliable picture of the annelids phylogeny and to establish an uniform systematics of these invertebrates (e.g. Marotta et al., 2008). In our study we considered the characters of the female reproductive system for phylogenetic relationships among Oligocheata *sensu lato*, it means oligocheates and leeches accordingly to traditional systematics.

Parsimony analysis included 29 representatives Oligochaeta *sensu lato*. Character set consisted of 18 features of the female reproductive system including ultrastructural ones. Phylogenetic analyses were performed in TNT version 1.1 (Goloboff et al., 2003) under a traditional searching with TBR branch swapping. Clade support was estimated with bootstrap option. The phylogenetic inference resulted in 40 most-parsimonious trees (length = 35). The strict consensus of these trees shows six well-supported clades: Naididae, Glossiphoniidae, Piscicolidae, Hirudiniformes, Erpobdelliformes, *Erpobdella*. Leeches classified in the order Hirudinida plus *Acanthobdella peledina* formed a monophyletic group. The relations among other taxa remained unresolved. Our results suggest that the characters of the female reproductive system seem to be quite conservative within Olichochaeta *sensu lato*. However, these characters appeared to useful for phylogenetic inference of leeches what is the confirmation of our previous study (Bielecki et al., 2014).

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Ploidy level and genome structure of polyploid *Cobitis* taxa (Pisces, Cobitidae) occurring in Poland. What are the benefits of polyploidy?

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This work summarized the results of comparative chromosomal studies of Cobitis taxa from 14 locations in the basin of four rivers and in two lakes. Most of them (nine) have not been previously karyologically described, whereas the rest have been verified.

Two distinct species, viz. the spined loach *C. taenia* (2n=48) and the danubian loach *C. elongatoides* (2n=50), four different triploid (3n=73; 3n=74; 3n=74*; 3n=75) and two tetraploid (4n=98; 4n=99) *Cobitis* forms were recognized among the samples. The populations of *C. taenia* were detected in two lakes and in the Łyna River whereas *C. elongatoides* in one river. All polyploid hybrids could not be distinguished by external morphology. The results showed that all mixed diploid-polyploid populations were dominated by triploid hybrid females, accompanied by a small number of *C. taenia* diploids and *Cobitis* tetraploids of both sexes.

The genome composition of polyploid taxa we verified using karyotype structure (Majtánová et al., 2016) and the microsatellites analysis (not presented here). The chromosome sets of 3n *Cobitis* females were composed of the *C. taenia, C. elongatoides* and *C. tanaitica* or *C. elongatoides* genomes. The chromosome pattern of tetraploids reflected their origin from triploid females by sexual reproduction with *C. taenia* males (the only fertile males among *Cobitis* taxa in mixed populations) (Juchno et al., 2014).

The presented data give an important insight into the genome compositions of polyploid *Cobitis* loaches complementing pattern of their distribution in Polish water bodies. The benefits of polyploidy are listed and discussed at the end.

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Studies on the embryo sac of *Sedum hispanicum* L. (Crassulaceae)

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In *Sedum hispanicum* the ovule is anatropous, bitegmic and crassinucellate. The development of the female gametophyte conforms to the Polygonum type. The embryo sac is forming within ovule as a result of mitotic divisions of functional megaspore. Our anatomical studies show that mature embryo sac is composed by seven cells. At the chalazal end of the young embryo sac are present three cells – well developed antipodal cells. At this time two polar nuclei are visible in central cell – the largest cell of the embryo sac with big central vacuole. At later stage of development this two polar nuclei contact, fuse and form secondary nucleus. In addition the antipodal cells are beginning degenerate. In mature embryo sac the egg apparatus (two synergids and egg cell) is located at micropylar pole. Starch granules are accumulating during development of the embryo sac. Zygote is formed after fertilization of the egg cell. It forms two-celled embryo as a result of asymmetrical division.

Androgenesis as a tool to broaden the genetic pool of common wheat

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The related species of the Poaceae family (Triticeae) are the source of unprecedented new genes (desirable for disease resistant, drought tolerance etc.) that allow the extension of genetic variation of common wheat (*Triticum aestivum* L.). These species contain both homeological chromosomes and rDNA sequences very similar to *T. aestivum* L. (Frederiksen and Seberg, 1992, Sasanuma et al., 2002, Zhang et al., 2008). Such a system allows the introgression of alien genes and their incorporation into the genomes A, B and D of wheat, where they can function permanently. Many of them have already been transferred to the varieties of *T. aestivum* L. (Pilch, 2011).

This study presents introgressive winter lines derived with the interspecific and intergeneric hybridization of *T. aestivum L.* with *T. durum* Desf., *T. timopheevii* Zhuk., *L. perenne* L. and *Aegilops speltoides* Taush. They were improved (in comparison to the cultivars and breeding materials) in some respects of both spike and grain characters.

The lines were developed using: (1) interspecific and intergeneric generative hybridization, (2) the genetic systems of wheat (*T. aestivum* L.) including the cross ability genes (*Kr*), and the *Ph* genes – homeology/homology pairing system (5B-chromosome system), and (3) positive selection directed on both spike and grain characters.

In view the opportunity to receive homozygotic lines possessing alleles of gene in recessive form, in a short time, the next step was to fix the improvements reached in the materials using the androgenesis system.

In total, 543 plants was regenerated by androgenesis process. The obtained lines showed improved connections of 7–11 features in 15 different combinations, giving breeders the ability to choose the line with the desired parameters.

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Anatomy of the Carpathian newt (*Lissotriton montandoni*) (Salamandridae, Urodela) spermathecae

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Salamanders are, in terms of breeding, one of the most interesting animals among vertebrates. Internal fertilization is common among majority of Urodela species, although during mating copulation does not takes place, since males do not possess copulatory organ. They deposit packs of sperm, called spermatophores, during mating, and the females collect the spermatophores with cloacal lips and store sperm for even few months in a special structure called spermathecae.

Lissotriton montandoni is a newt species, which possess a simple type of spermathecae. This type consists of numerous tubular glands, individually opening into the roof of the cloaca. The tubules are lined with cubic epithelium, while the inner walls of the cloaca are lined with columnar epithelium. Numerous glandular, mucus-producing cells are present in the epithelium. The space between the tubules of the spermathecae is filled with connective tissue and contains melanophores, blood vessels, collagen and elastic fibers.

The results of our study showed that the structure of the Carpathian newt spermathecae does not differ much from those of other salamanders with simple spermathecae. *L. montandoni* exhibits the greatest similarity in anatomy of spermathecae to closely related species, the smooth newt (*L. vulgaris*). However, some differences were found between these two species, including differences in the composition of the mucus secreted by the spermathecae tubules. This phenomenon could potentially affect the lower interspecies mating success between *L. montandoni* and *L. vulgaris*.

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Anatomy of the female reproductive system of the caecilian *Typhlonectes natans* (Fischer In Peters, 1880) (Amphibia: Gymnophiona)

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Caecilians (Gymnophiona) remains the least researched clade of amphibians. Members of this group live in tropical regions of southern hemisphere, under the ground or in the water. These animals with internal fertilization are viviparous or oviparous. Males posses an introminent organ called a fallodeum.

Typhlonectes natans (family Typhlonectidae) is an aquatic, viviparous species, which lives in the Cauca and Magdalena rivers in Columbia and Lake Maracaibo in Venezuela. Its body length is from 45 to 55 cm. In our research we described for the first time the anatomy of female reproductive system of *T. natans*. We used two specimens. Our observations were made using light microscopy and compared with available literature data.

The female reproductive tract consists of paired ovaries, accompanied by twisted fat bodies, long paired oviducts, which are divided into two distinct regions – anterior and posterior, and the cloaca.

In ovaries, arranged in a segmental manner, numerous oocytes at different stages of development (including previtellogenic, vitellogenic and postvitellogenic) were present. The space between oocytes was filled by connective tissue. The ovaries were covered by connective tissue and enveloped by peritoneal epithelium.

The anterior part of oviduct exhibited large lumen and relatively thin layer of muscles in contrast to the posterior part (so called "pars uterine"), which had a thick layer of muscle and lower lumen. Oviduct wall consists of several layers, with the mucous membrane lining the lumen, a layer of smooth muscles and the external serous membrane. In epithelium we observed a few wedge-shaped cells. The presence of this cells was not previously reported in mucous membrane of the oviduct in any caecilian. We also observed histological differences in composition of the mucous in the lumen of the cloaca in comparison with previously described species.

Despite the females have ovulated eggs, no sperm were detected in any part of oviducts and cloaca. The question of eventually sperm storage in viviparous species of caecilians is still unresolved.

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The embryonic development of nematodes, genus *Nematodirus*

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There are two types of ovaries which occur in nematodes: telogenic and hologenic ovaries (Bird, 1991). Egg cells in telogenic ovaries are produced in the initial section of the ovary. Cells of germinal epithelium, situated in the section, undergo several divisions and produce oogonia, connected with the ovary with a plasmatic projection used for feeding. In the farther part of the ovary, oocytes detach themselves from the wall and adopt a spherical shape. The germinal epithelium in hologenic ovaries is situated on the entire internal wall of the ovary. After they stop growing, eggs detach themselves from the ovary wall and move to the fallopian tube (Marquardt et al., 2000).

The aim of the study was to explore the embryonic development of nematodes, genus *Nematodirus*.

The nematodes isolated from the small intestine of deer were fixed in 70% alcohol and cleared in glycerol. The observations revealed that ovaries in the nematodes in genus *Nematodirus* are of the hologenic type. Oogonia are not formed on the entire internal wall of the ovary, but only in the dorsolateral area. After their growth is completed, oocytes detach themselves and move to the abdominal part of the ovary and then to the oviduct tube. Immediately after fertilisation, the embryonic development stage (cleavage and gastrulation) begins.

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Two types of germ cell clusters in the ovaries of podocopid ostracods

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In invertebrates two basic types of the ovaries have been described i.e. panoistic and meroistic. In panoistic ovaries all germline cells have the ability to develop into functional oocytes. In meroistic ovaries germline cells are connected by intercellular bridges and cell clusters. Within the cluster the germ cells differentiate into the oocytes and nurse cells.

Extensive comparative investigations revealed that germline clusters may differ significantly from each other. Differences concern features such as: a total number of the cystocytes in the cluster and also a number of the oocytes per cluster. In most cases only one cell in the cluster becomes the oocyte. Such clusters are known from some annelids, crustaceans and insects. The germ cell clusters comprising more than one oocyte are relatively rare and have been described in some annelids and some mites. It has been also demonstrated that the cells in the cluster may be arranged in different ways: linear or ramified. The linear germ cell clusters have been described in polychaetes, some crustaceans and endopterygote hexapods.

In most investigated crustaceans the ovary is of panoistic type, whilst meroistic ovaries are known only from Branchiopoda and Podocopa. It has been previously found that in crustaceans germ cell clusters are two-cell or multicellular. The latter exhibit linear arrangement. In studied podocopid ostracods, the occurrence of two types of germ cell clusters has been revealed. In *Ilyocypris bradyi*, *Cyclocypris ovum* and *Notodromas monacha*, the germ cell clusters consist of two cells that diversify into the oocyte and nurse cell. In *Cypris pubera*, the ovaries contain multicellular germ cell clusters of unusual construction. Namely, the germ cell clusters are composed of several oocytes connected by means of cytoplasmic bridges to one, centrally located nurse cell. Usefulness of features associated with the structure of ovaries in phylogenetic analysis within Ostracoda-Podocopa will be verified.

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The ovule and embryo sac development at *Tinantia anomala* (Commelinaceae)

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Tinantia anomala is an interesting model of embryological investigations but there are few papers describing the biology of this species. The first paper on biology of this species has been published by Vogel in 1978. He reported about mimicry *T. anomala* flowers but microsporogenesis and megasporogenesis were not investigated at all. Flower of a zygomorphic symmetry with morphologically diverse anthers is of a particular interest. The female gametophyte plays a critical role in essentially every step of the reproductive process. The female gametophyte participates in directing the pollen tube to the ovule and upon fertilization, female gametophyte controls the initiation of seed development.

The ovules in *T. anomala* developed in a 3-locular ovary. The campylotropous ovules had two integuments and a thick nucellus and they were attached to the placenta with a very thick funicle. They were composed of a nucellus surrounded by two integuments; the outer integument consisted of 4 cell layers and the inner one was thinner and had two cell layers. Anatomical analysis showed two zones in the ovule delineated by the inner integument growing into the nucellus. The micropylar part of the ovule was composed of few parenchyma cells, whereas the chalazal part was strongly developed and several times larger than the micropylar part. The ends of the integuments formed a characteristic, zigzag micropylar channel. At the micropylar pole of the nucellus, an archesporial cell differentiated and passed asymmetric mitosis resulting in a subepidermally located smaller cover cell and a larger megaspore mother cell located more deeply. The megasporocyte underwent meiotic division, and 4 haploid daughter cells arranged in a megaspore tetrad were formed. The cell located at the micropylar pole was an active megaspore, whereas the other 3 megaspores degenerated. The gametophyte in *T. anomala* represented monosporic the Oenothera type. A filiform apparatus was observed in one of the synergids.

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Organisation of the egg capsule of *Macrobiotus pallari* and *Richtersius coronifer* (Tardigrada, Eutardigrada, Parachela)

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Organisation of the egg capsule of two species of tardigrades belonging to the family Macrobiotidae, *Macrobiotus pallari* and *Richtersius coronifer*, was analysed using light, transmission and scanning electron microscopy. In both cases, similar to other Macrobiotidae (Węglarska, 1982; Poprawa et al., 2015) egg capsule was composed of thin vitelline envelope and three-layered chorion. The chorion consisted of: (1) the inner, medium electron-dense layer – endochorion, (2) the middle, labyrinthine layer and (3) the outer, medium electron-dense layer – exochorion.

The yellow chorion of *Richtersius coronifer* was covered with elongated processes having irregular granulation on their surface. These processes were empty inside. They were formed by evagination of exochorion. The basic chorion connecting processes was smooth and covered with irregular granulation.

The chorion of *Macrobiotus pallari* was white. Its surface was covered with conical processes and areolation between them. The tips of processes were elongated and formed bushy structure. The surface of conical processes has a form of sculpture composed of thin rings. The processes that were empty inside, were formed by evagination of exochorion and labyrinthine layer.

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Effect of directional selection on relation between egg and early embryo traits in Japanese quail (*Coturnix japonica*)

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Body weight reached in a certain age is the main criterion for selection in poultry meat production. Egg weight is one of the factors playing an important role in the development of embryos and chicks hatchability, but the observed differences also depend on the line genetic background. Japanese quail can be used as an animal model in many areas of science, because of their advantages.

The aim of this experiment was to determine the relation between egg weight and different early embryo traits in Japanese quail selected for the 28th day body weight.

Depending on the studied traits there were analyzed in total from 584 to 670 Japanese quail eggs obtained from 75 parental pairs of 10th generation of experimental groups (K – control and S – intra-family selection on 28th day body weight). All eggs were weighed and stored in 16°C (±1°C) for not longer than 24 hours to avoid uncontrolled germinal cells division (Grzegrzółka and Michalska, 2008). Incubation was carried out in incubator with automatical egg turning every hour, in 37.8–37.9°C of temperature and 65% of relative humidity. Morphological observations of embryos development and their measurements were taken after 24 and 48 hours of incubation. Applied embryo staging system based on Zacchei (1961) and Hamburger and Hamilton (1951) classifications (Grzegrzółka and Michalska, 2008). Statistical analysis was carried out using SPSS 12.0 PL package.

Estimated phenotypic correlation coefficients between the egg weight, stage of development and other selected traits observed in embryos after 24 and 48 h of incubation remained at low level, and not all were statistically significant. An exception were quite strong stage correlations with blastoderm diameter and surface area after 24 h ($r>0.61$), and vascular surface area after 48 h ($r>0.69$) estimated in both groups. Results obtained in earlier studies on quails (Korzyńska-Nowak and Michalska, 1992/93) also indicated a low or lack, and even a slight negative correlation between egg weight and the rate of early embryonic development, but they were not focused on selection factor.

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Early embryo development in relation to egg weight loss in a different coloured eggs of ring-necked pheasant (*Phasianus colchicus*)

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Ring-necked pheasant plays an important role as the game bird. To avoid excessive reduction of wildlife populations it is necessary to supplement them with farm-bred birds. Pheasant females lay eggs with a different shell colour that may be related with egg quality and embryo development characteristics (Kirikçi et al., 2005; Kożuszek et al., 2009).

In total, 68 pheasant embryos of four groups of shell colours were analysed in experiment: 15 blue, 16 dark-brown, 19 light-brown and 18 olive. All eggs were collected the same morning and not stored to avoid uncontrolled embryo development as the additional effect (Grzegrzółka and Michalska, 2008). The incubation was carried out for 72 hours in 37.5°C of temperature and 65% of relative humidity, with automatical egg turning every hour. After incubation and blastoderm preparations the morphological evaluation of 72 h old embryos according to the chicken embryo development staging by Hamburger and Hamilton (1951) was conducted.

Egg weight loss (%) observed during first 72 h of incubation was significantly higher in olive coloured eggs than in dark- and light-brown ones. Blue eggs reduced their weight in moderate rate, not different statistically from the other groups. Higher egg weight loss of olive eggs did not affect significantly the embryo and extra-embryonic membranes development. All parameters, like area vasculosa diameter, area pellucida length, embryo stage and length, number of somites pairs and amniotic fold development reached even slightly higher values in olive and blue coloured eggs than in the other groups.

This may indicate the need for adjustment of incubation conditions to reach the same satisfactory level of hatchability in each eggshell colour group.

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The length of cervical, thoracic and lumbar regions of vertebral column in 7th and 8th week of embryonic development

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From data presented by Jackson (1909) it can be observed that at the end of embryonic period cervical region of vertebral column, comparing it to earlier stages, starts to decrease, lumbar tends to increase and thoracic remain the same. Bagnal (1979) shown that from 8th to 26th week of fetal development the thoracic region is always longest, lumbar region is longer than cervical.

The aim of the study was to compare the length of cervical, thoracic and lumbar regions of vertebral column in 7th and 8th week of embryological development.

Study was performed on 15 human embryos from the Collection of Department of Anatomy, University of Medical Sciences in Poznań. Age of embryos was determined according to 23 international Carnegie stages and was expressed in post-fertilizational days. Embryos were embedded in toto and sectioned in sagittal, frontal and horizontal planes. Serial sections were stained with various histological methods and impregnated with protargol (Bodian method) and silver gelatin using the method of Pearson and O'Neil. Regions of vertebral column were measured with Axio Vision LE computer program.

Vertebral column develops from paraxial mesoderm called somites. In stage 13 somites begin to divide into dermatomes, myotomes and sclerotomes.

In the 6th week of fetal development (stage 17) loose and dense zones of cells in the sclerotomes are distinguishable. The vertebrae develops from loose zones as the dense zones forms future intervertebral discs. At the beginning of 7th week the approximate length of regions of the vertebral column are: cervical: 1,4 mm, thoracic: 2,1 mm, lumbar: 1,2 mm.

In the 7th week of development (stage 18) thoracic region of the vertebral column is longest. Lumbar region is not well developed and is shortest.

At stages 19 and 20 the vertebral column grow rapidly. Discrepancy between length of cervical and lumbar region becomes smaller. The notochord become segmented.

During the 8th week of development (stages 21, 22) length of thoracic region of the vertebral column does not change. At stage 23 approximate length of regions of the vertebral column are: cervical 4 mm, thoracic 8,8 mm and lumbar 4 mm.

In last week of embryonic period the X, Y and Z zones in superior two cervical vertebrae can be distinguished. From the zones X and Y develop the dens of axis, and from the zone Z the body of C2.

In two last weeks of embryonic development (stages 18–23) thoracic region of the vertebral column is longest, the both cervical and lumbar regions are similar in length.

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Studies on autogamy in *Epipactis helleborine* (L.) Crantz. (Orchidaceae)

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Orchids reproduce mainly by cross-pollination. However, when grown in an environment where there is a limited amount of pollinators (e.g. the dark forest, seaside coast), some species of orchids are capable of reproduction by autogamy. Autogamy is a form of self-pollination, in which a flower is fertilized by pollen from the same flower. According to Catling (1990) 5–20% of species in the Orchidaceae are able to reproduce by autogamy.

The research was conducted on *Epipactis helleborine*, in natural habitat (mixed forest of Sopot Kamienny Potok). This species grows on fertile beech forests, coastal dunes and meadows and also in the environment created by man, e.g. roadside, ditches. It blooms from June to September. Seven inflorescences were bagged with nylon mesh just before anthesis to prevent outer/external pollen transfer on the stigma. Flowers were hand-pollinated by pollen from the same flower (induced autogamy). Flowers were collected at 24 hours, 36 hours, 48 hours, 4 days, 8 days after hand-pollination. The plant material was fixed, paraffin-embedded, sectioned and stained with alcian blue and haematoxylin. Longitudinal sections of the flowers were analyzed using the light microscope.

Pollen tubes were observed in the stylar canal and ovary. Stages from mature embryo sacs to young embryos were present inside ovaries. Thus, success in embryogenesis was found. These studies proved that the *Epipactis helleborine* is capable to autogamy. These anatomical results confirm previous ecological studies conducted in Biebrza National Park and Wigry National Park (Tałałaj et al., 2008). Knowledge of autogamy is useful to explain the success of this species in the colonization of new habitats and can be used for species protection.

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Microstructure of gustatory lingual papillae in the donkey during prenatal period

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The gustatory papillae on the tongue develops during prenatal period prior to mechanical papillae. This observations was described so far in some laboratory rodents like rat and mouse and in domestic cat. The aim of our histological and scanning electron microscopic observation was to describe the distribution of primordia of fungiform papillae and vallate papillae and their microscopic structure.

The study was carried out on fetuses of donkey from 100–350 days of pregnancy. The tongue has been removed from fetuses and fixed in 10 % formaldehyde. For the microscopical observations of the dorsal surface of the tongue in the scanning electron microscope the tissues were dehydrated and critical-point dried, coated with gold layer and observed in scanning electron microscope ZEISS 435 VP at 15 kV. Probes from apex, body, prominence and root of the tongue were routinely processes for preparation of histological slides stained with Masson-Goldner trichrome.

Our observation revealed the at ca 130–138 day of pregnancy the on apex and body of the tongue evenly distributed primordia of fungiform papillae were present. Two or three oval primorida of vallate papillae are situated in one row on caudal part of dorsum linguae.

Between 201 and 208 day of pregnancy evenly distributed fungiform papillae rise over the mucosal surface of apex and body of the tongue. The vallate papillae posses a distinct body of papillae.

At 313–320 day fungiform papillae are distributed on apex, body and on lateral surfaces of the lingual prominence. Around body of vallate papillae clefts appear. The filiform and conical papillae are rounded primordial structures.

In fetuses aged on 313–320 day p.c. the median sulcus extends from apex to root of the tongue. Fungiform papillae are distinct structures on apex, body and lateral surfaces of the lingual prominence. Around two or three vallate papillae, situated on median line of the anterior part lingual root, continuous furrow and flat pad is observed.

Cytoarchitecture of oocytes of the red claw crayfish, *Cherax quadricarinatus* (Crustacea, Malacostraca: Decapoda)

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The mature ovary of *Cherax quadricarcinatus* is sac-shaped and filled with oocytes at previtellogenic, vitellogenic and choriogenic stages of oogenesis. Such an ovary organization indicates asynchronous development of the female germ cells. Throughout oogenesis, a spherical nucleus, or germinal vesicle, resides in the center of the oocytes. The nucleus is surrounded by a regular nuclear envelope pierced with numerous nuclear pores. The nucleoplasm contains 2–3 prominent nucleoli and dozens of smaller nuclear bodies. The most interesting feature of the oocytes is the architecture of their cytoplasm, or ooplasm. In the early previtellogenic oocytes, the ooplasm is homogenous, however in the late previtellogenic and early vitellogenic oocytes, it differentiates into three morphologically distinct and concentric zones: perinuclear, main and cortical. Such heterogeneous organization of ooplasm is unusual and to our knowledge has not been reported in oocytes of decapod crustaceans before (for review see Krol et al., 1992; Lopez-Greco, 2013). The perinuclear zone is devoid of membranous organelles and, as extraction with Triton revealed, contains an intricate network of microtubules. This network may be responsible for maintaining a central position of the oocyte nucleus, but it may also participate in the microtubule-based transport of macromolecules, most likely of the nucleolar origin, from the perinuclear region towards the periphery of the oocyte. The main cytoplasm is filled with centrally located yolk spheres interspersed with small lipid droplets, whereas large lipid droplets are distributed more peripherally. The ooplasm heterogeneity disappears in the late vitellogenic oocytes, as they become filled with the reserve materials. The oocytes are surrounded by somatic follicle cells forming a simple epithelium. Throughout oogenesis, the follicle cells remain morphologically uniform. A single, simple egg envelope is deposited in the perioocytic space, i.e. between the oocyte membrane and the follicular epithelium.

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Callose deposition during megasporogenesis in two diploid *Hieracium* species

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Callose is a polysaccharide deposited in the walls of various plant cell during a wide variety of biological processes including cytokinesis, pollen tube growth, responses to abiotic and biotic stress, and reproductive development. It is believed that callose is a cytological marker of meiocytes in both the anthers and ovules. Rodkiewicz (1970) was the first who described the pattern of callose deposition during megasporogenesis in some angiosperms. Since then, the localization of callose has been analyzed in the ovules of other sexual, as well as apomictic species. Despite much progress in the research on callose deposition, its role and the molecular mechanisms of its synthesis during megasporogenesis are still poorly understood.

The aim of this study was an analysis of callose deposition in young ovules of diploid *Hieracium transylvanicum* and *H. pavichii* (Asteraceae). Observations of ovaries cleared in methyl salicylate revealed the presence of a single archesporial cell in the ovules of both species. This cell developed directly into a megaspore mother cell (MMC) and a linear tetrad of megaspores formed after its meiotic division. The three micropylar megaspores degenerated, while the chalazal one developed into a functional megaspore (FM). Aniline blue staining was used to the detect of callose. Lack of callose in the wall of the archesporial cell was a feature common to both of the studied species. Fluorescence of callose was noticeable at the stage of prophase I. Surprisingly, despite the same course of megasporogenesis, our observations revealed different patterns of callose deposition in the ovules of both species. At the very early stages of meiosis, in *H. transylvanicum* callose was visible only at the chalazal pole of the megasporocyte, whereas in *H. pavichii* it was observed at the micropylar pole of MMC. In subsequent stages of the megasporogenesis, callose distribution pattern was similar in both species. Prominent callose wall enveloped the MMC and after the first meiotic division, an intensive fluorescence of callose was visible in the newly formed transverse wall between the cells of the dyad, whereas the side wall showed a very weak fluorescence. In the young tetrad, the side walls were almost completely devoid of callose but it was present in the walls between the megaspores and the fluorescence signal was particularly strong between subchalazal and submicropylar cells. At this stage, callose also persisted at the chalazal pole of FM. Subsequently, this florescence signal disappeared and the intensive fluorescence of callose appeared around the degenerated megaspores.

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Structure and ultrastructure of the ovary in *Thulinius ruffoi* (Eutardigrada, Parachela, Isohypsibiidae)

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Thulinius ruffoi (Parachela: Isohypsibiidae) is an exclusively aquatic species that reproduces parthenogenetically. The specimens were collected in the wastewater treatment plant in Zelków, near Cracow and reared in the laboratory. The structure and ultrastructure of the ovary of *Thulinius ruffoi* were analysed using light and transmission electron microscopy. The ovary of the species examined, similar to other eutardigrades (Poprawa, 2005; Poprawa et al., 2015a, b), was composed of two parts: small germarium and bigger vitellarium. The wall of the ovary was formed by the single flat epithelium suspended by the basal lamina. The germarium was filled with the oogonia that divided mitotically, while the vitellarium was filled with clusters of the germ cells. The germ cell clusters had shapes of rosette. The cells in each cluster were connected by the intercellular bridges that possessed one electron-dense rim suspended to the plasma membrane. One cell in the each cluster developed into the oocyte while the remaining cells became trophocytes (nurse cells). The main function of the trophocytes were synthesis of rRNA and reserve material, and distribution them to the oocyte.

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Ovary cord organization in *Haemopis sanguisuga* (Annelida, Clitellata)

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Haemopis sanguisuga (a horse leech) is a cosmopolitan freshwater leech. Fully mature specimens can be up to 10 cm in length. This leech, which is carnivorous, usually feeds on small invertebrates.

The female reproductive system of *H. sanguisuga* is composed of paired ovaries and reproductive tracks. Each ovary is built from the outer somatic envelope (coelomic epithelia, connective tissue and muscles), the so-called ovisac, forming a space (the ovisac lumen) that is filled with coelomic fluid. The germ cells within the ovisac are interconnected and form syncytial cysts. Clustered germ cells together with somatic cells form long and strongly convoluted structures known as ovary cords. In the studied species, there are always two ovary cords within each ovisac. In addition to the ovary cords within the ovisac, lumen vitellogenic oocytes and coelomocytes can be found. Three morphologically different zones can be distinguished in each ovary cord. One end of the cord, regarded as "apical", is club-shaped and consists of germ cells (oogonia and entering meiosis cystocytes) and somatic cells intermingled between the germ cells. One somatic cell, which is located at the top of the club-shaped end and is known as the apical cell, is especially large and forms long cytoplasmic projections. The middle zone of the cord houses two subpopulations of germ cells – oocytes, which grow and gradually protrude from the cord into the ovisac lumen, and numerous but small germ cells, which are regarded as trophocytes. The growing oocytes eventually detach from the cord and float freely in the ovisac lumen. The third zone (the distal end of the cord) contains degenerating trophocytes and numerous somatic cells.

The presented studies, the main goal of which is to analyse the organization and functioning of the cytoskeleton in developing germ-line cysts and to shed more light on the structure and function of the peculiar somatic cell, the apical cell, are preliminary.

Fine structure of the degenerative cells of tetraploid *Cobitis* (Pisces, Cobitidae) testes

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Most of the *Cobitis* populations in Poland are mixed diploid-polyploid, composed of diploid and/or polyploid hybrids co-exiting at least with one of their parental species (*Cobitis taenia* and *C. elongatoides*) whose males participate in the reproduction of the unisexual forms. Bisexual tetraploid loaches are resulted from hybridization between diploid bisexual males and triploid females. The results of previous histological data revealed the lack of spermatozoa in the testes of tetraploids (Juchno and Boroń, 2006).

The aim of the studies was the ultrastructure of degenerative cells of the testes of tetraploid *Cobitis* from diploid-tetraploid populations in Pilica and Kortówka Rivers (Poland) presented here for the first time.

The testes cysts contained only correctly developed spermatogonia A and B, and spermatocytes with synaptonemal complexes and Sertoli cells. However, there were no cysts with correct spermatids and spermatozoa. Some of the degenerative cells were characterized by swollen mitochondria, abnormally large crescent-shaped mitochondria and dilated smooth endoplasmic reticulum. In the cytoplasm of degenerative germ cells most commonly were observed autophagosomes (or autophagic vacuoles) containing myelin-like organelles and also large vacuole-like structures, multilamellar bodies and cytoplasmic particles. Additionally, degenerating (most likely apoptotic) cells were characterized frequently by a ring-like condensation of chromatin around the nuclear periphery or in the central part of nucleus. Strong electron-dense material of irregular shape resulting from defragmentation of chromatin indicated the beginning of the degenerative process was detected in some of germ cells. The degeneration process concerned a single or all of the cells within the cysts. Various stages of cell degeneration within cysts commonly were observed. Sometimes all cells have undergone lysis but the boundaries of cysts remain unchanged or some of germ cells were separated from Sertoli cells.

The reasons of degenerative changes in the testes and sterility of tetraploid Cobitis males are discussed and they are not unambiguous and doubtless arise from the function of genes controlling the process of reproduction.

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Changes in the salivary glands of the *Scolopendra cingulata* (Myriapoda, Chilopoda, Scolopendridae) during starvation

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Scolopendra cingulata is the biggest European species of centipedes and the smallest one among Scolopendridae. It lives under stones, leaf litter and rocks, and prefers dark and high humidity environment. The digestive system of this species is composed of three distinct regions: foregut, midgut and hindgut. The paired salivary glands located in the neighborhood of foregut, are irregular in shape. Salivary glands, as organs which belong to alimentary tract, play an important role in preliminary digestion, lubrication of food, elimination of pathogens and harmful materials that originate from food. Therefore, it treated as the good organ for analyzing all changes and alterations which occur according to any external stressors. Salivary glands of *S. cingulata* are covered by irregular fat body tissue and tracheoles. Each gland is formed by the glandular epithelium, which possesses two types of cells: the secretory cells I and II. Substances produced by these cells are released into conducting canal system. The fat body is composed of one type of cells which are responsible for the accumulation of the reserve material.

Adult specimens of *Scolopendra cingulata* (Myriapoda, Chilopoda) were prepared for the analysis with the use of confocal and transmission electron microscopy to visualize changes in salivary glands caused by starvation. Additionally, histochemical and immunohistochemical methods were established in order to describe which type of the reserve material is exploited and which type of the cell death takes part in the proper functioning of described above organs.

Fine structure and histochemical studies on salivary glands and fat body in centipedes (Myriapoda, Chilopoda)

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Centipedes (Chilopoda) are treated as important members of soil macrofauna due to their main role in biodegradation and fragmentation of dead plant material and other organic matter. Therefore, they are treated as bioindicators of the natural environment (Shear and Edgecombe, 2010).

As the material for the study, we used species that are easy to collect and/or easy to raise under laboratory conditions: *Geophilus flavus, Clinopodus flavidus, Lithobius forficatus* and *Scolopendra cingulata*. The salivary glands were investigated using light and transmission electron microscopes. Each gland is formed by the glandular epithelium, which possesses only one type of cells: the secretory cells. The ultrastructure of secretory cells suggest their role in the synthesis and secretion of substances. The organs are surrounded by fat body tissue, which forms the irregular mass. Fat body cells of *L. forficatus* have many granules of pigment, which makes it the purple in colour. The cytoplasm of fat body is poor in organelles. All substances produced by secretory cells of the salivary glands are realeased into the conducting canal system. Salivary glands of *G. flavus* and *C. flavidus* are tubular in shape. However, organs of *L. forficatus* and *S. cingulata* have a vesicular structure and are composed of numerous large lobes and vesicles.

Histochemical stainings revealed the the material accumulated in the cytoplasm of the secretory cells in salivary glands are polysaccharides, mucopolysaccharides and proteins.

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Effects of inhibitors upon the division of generative cell in *Gagea lutea*

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Gagea lutea (Yellow star-of-Bethlehem) is a perennial bulb geophyte belonging to the lily family and widespread in deciduous woodlands of Eurasia (Yoshie, 2008). Among plants, *G. lutea* has the biggest sperm cells second only to *Zamia* (Cycadophyta; Southworth and Cresti, 1997). Generative cell (GC) of this species has an abundant microtubules bundles surrounding nuclei in germinating pollen tube. Finally, mature male gametophyte of Yellow star-of-Bethlehem is consisted of highly dimorphic sperm cells, the first sperm cell is always followed by the second one, which is nearly three times bigger (Zhang et al., 1995). All of these together make it a perfect model for experiments regarding dynamics of second pollen mitosis (PMII) among monocots. Mitotic spindle and phragmoplast formation are critically dependent upon proper organization of cytoskeleton. Here we show the impact of selected concentrations of paclitaxel, oryzalin, cytochalasin D and Latrunculin B, focusing on the symmetry of generative cell division of *G. lutea*. First of the two used agents interfere with microtubules dynamics which resulted in newly formed symmetrical sperm cells in contrast to SC observed in natural conditions. The last two toxins did not affect the generative cell division as they are believed to disrupt microfilaments which are absent in GC and SC in Yellow star-of-Bethlehem.

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The most probable explanation of decreased pollen fertility in *Rumex hastatulus* hybrids (Texas x North Carolina)

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Rumex hastatulus, a representative of the section *Americanae* in the genus *Rumex*, is the only known dioecious plant with two sex chromosome systems: XX/XY in the Texas (T) race (2n=10) and XX/XY1Y2 in the North Carolina (NC) race (2n=8, 9) (Smith, 1963). Co-existence of two different sex chromosome systems within one species, as well as their relatively young age (ca. 600,000 years) create a unique opportunity to track the early stages of evolution of the XX/XY1Y2 system in plants.

C-banding/DAPI method, FISH with 5S and 35S rDNA and flow cytometric studies suggested that XX/XY1Y2 system of the NC race originated from the XX/XY system of the T race through the chromosome translocations accompanied by dysploid reduction of chromosome number and genome downsizing (Grabowska-Joachimiak et al., 2015). It created three neo-sex chromosomes in the NC race: 1) the X_n chromosome composed of the original X chromosome and a large acentric fragment of the 3rd T autosome (3Ta), 2) the Y1 chromosome representing large centric part of the primary Y chromosome, 3) the Y2 chromosome consisting of the 3Ta and acentric fragment of the primary Y chromosome.

To confirm this hypothesis we analyzed microsporogenesis in experimental F1 hybrids between races of *R. hastatulus* (T x NC and NC x T). According to translocation hypothesis, meiosis in male F1 hybrids should proceed without disturbances. NC x T hybrid should form three autosomal bivalents + Y-X_n-3Ta trivalent, while T x NC hybrid should form 5 bivalents (three autosomal + X-Y1 + Y2-3Ta). These predictions were fully confirmed by us.

Because F1 hybrids were available, we perform additional analysis of pollen fertility in them. It turned out that they substantially differed in this respect: pollen grains of NC x T hybrids showed high fertility (>90%), whereas in T x NC hybrids only 70% of pollen grains were fertile. Most probably this reduction of pollen viability is caused by the occurrence of only one Y chromosome in 1/4 of microspores in T x NC males. The rest of microspores possess the X chromosome or both Y1 and Y2 chromosomes (= equivalent of the original Y chromosome). It shows that partially heterochromatinized Y chromosomes of *R. hastatulus* are still equipped with indispensable genetic material.

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Ultrastructure, distribution and transovarial transmission of symbiotic microorganisms in a leafhopper *Graphocraerus ventralis* (Insecta, Hemiptera, Deltocephalinae)

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Graphocraerus ventralis is a plant sap-feeding leafhopper. Since its diet does not contain essential amino acids, missing components are supplied by intracellular symbionts (bacteria or yeast-like symbionts). Symbiotic microorganisms are localized in the cytoplasm of giant, polyploid cells called bacteriocytes (if the symbionts are bacteria) or mycetocytes (if the symbionts are yeast).

Leafhoppers living in Hemiptera: Cicadomorpha are as a rule hosts for two bacterial species. Since results of molecular analyzes have shown that all these bacteria are involved in the synthesis of essential amino acids, symbionts of leafhoppers have been called "coprimary symbionts" (Takiya et al., 2006). It is believed that the common ancestor of these bugs has been infected by the bacterium *Sulcia* (phylum Bacteroidetes) and bacterium belonging to the class Betaproteobacteria (phylum Proteobacteria). During further evolution of some lineages the betaproteobacterium has been replaced by other bacteria (Koga et al., 2013) or yeast-like symbionts (Sacchi et al., 2008).

Histological, ultrastructural and molecular analyses revealed that in the body of *G. ventralis* bacterium *Sulcia* and yeast-like symbionts occur. The bacteria *Sulcia* are harbored in the cytoplasm of bacteriocytes, whereas yeast-like symbionts are distributed in fat body cells. This is a unique situation, because such a system symbiotic (*Sulcia* + yeast-like symbiont) has never been reported for leafhoppers.

Both types of symbionts are transovarially transmitted to the next generation. They migrate from the bacteriocytes/fat body through the follicular cells to the perivitelline space, where they form "a symbiotic ball" in the deep invagination of oolemma.

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The *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE* gene is differentially expressed between cells types and their developmental fate in *Trifolium nigrescens* cultured *in vitro*

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The *SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE* (*SERK*) gene encodes a transmembrane protein belonging to the family of leucine-rich repeat receptor-like kinases (Hecht et al., 2001). *SERKs* from different plant species were shown to be involved in a wide range of physiological processes like adaptation to environmental stresses, induction of cellular totipotency or sexual reproduction. More recently, the involvement of these genes in *de novo* organogenesis has also been shown (Du et al., 2011). The multifunctionality of *SERKs* implies a need for a detailed expressional analysis of each new identified gene to properly determine its function in particular plant. Previously, we identified the first among clovers *SERK*s orthologue from *Trifolium nigrescens* Viv. (*TnSERK*) (Pilarska et al., 2016). We revealed the engagement of *TnSERK* in induction and maintenance of embryogenic potential in tissues cultured *in vitro*.

In this study we used *in situ* hybrydization of RNA and real-time PCR to investigate the expression pattern.

In situ hybridization showed that the *TnSERK* was mainly expressed in actively dividing cells of callus from which meristemoids, roots or vascular strands were produced. Strong hybridization signal was also observed in meristematic cells of root meristem but it was gradually lost when the cells, matured and differentiated into non-dividing parenchyma and vascular elements of root/callus.

As revealed by real-time PCR both expression level of *TnSERK1* as well as the frequency of morphogenesis were strongly reduced by propiconazole indicating the involvement of brassinosteroid signaling in *TnSERK*-dependent developmental pathways. The relationships between *TnSERK* expression level, hydrogen peroxide content and polar auxin transport were also confirmed.

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Sedum as model genus for embryo/maternal tissue interaction studies

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Sedum, the largest genus (ca. 500 species) of Crassulaceae, has cosmopolitan distribution and shows much of the morphological diversity present in this family as a whole. Sedum undergoes the Caryophyllad type of embryonic development. The basal cell produces haustorial branches invading the micropyle and adjacent tissues, and protrudes out of the ovule. The endosperm forms a uninucleate haustorium at the chalazal pole. The embryogenesis in *Sedum* has been studied extensively at an ultrastructure level as model for suspensor physiology and function studies. The haustorial suspensor in particular its basal cell, undergoes developmental changes during embryogenesis. It is suggested that some, if not all of these changes are connected with the physiological requirements of the developing embryo. Ultrastructural analysis and cytochemical tests (including proteins, insoluble polysaccharides and lipids) indicate that in *Sedum* the embryo suspensor is involved mainly in the absorption and transport of metabolites from the ovular tissues to the developing embryo proper via the chalazal suspensor cells. The suspensor functions as a channel for transport of nutrients to the developing embryo proper from the surrounding maternal tissues – this is confirmed by the presence of plasmodesmata in the chalazal-end wall of the basal cell and in the end walls of the suspensor. The exchange of substances between the embryo and maternal tissues of may occurred through the suspensor via plasmodesmata and/or wall ingrowths resembled of transfer cell. An important question in plant embryology is the role of communication between different parts of the developing embryo. Plasmodesmata have been found between the embryo suspensor and the endosperm cells. Our recent studies in *S. acre* suggest that the symplasmic communication between *Sedum* seed compartments and the embryo and within the embryo change during the development.

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Morphometric characteristics of thyroid gland in postembryonic development of Anura

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In the processes of premetamorphosis and metamorphosis of Anura the level of hormones plays a crucial role, and their concentration change in subsequent stages of development (François-Krassowska, 1986). To demonstrate this, we compare the morphological parameters of thyroid gland in eight species of Anura, which belong to three families: Ranidae, Pelobatidae and Bufonidae. The object of the research were: *Rana esculenta, Rana bedriagae, Rana lessonae, Rana ridibunda, Pelobates fiscus, Bufo calamita, Bufo viridis* and *Bufo bufo*. In research taken into account two parameters describing relationship between the body size of tadpoles and the size of their thyroid gland. The first was the volume of secretory part of thyroid gland per gram of body weight of the tadpoles. The second was the total area of thyroid follicles per gram of body weight of the tadpoles. Both parameters were analyzed at stages II,VI,X, XVII of prometamorphosis and XXI, XXII of metamorphosis. The stages of development were distinguished after (Rugh, 1951).

Studies have shown that among species: *R. bedriage, R. lessonae, R. ridibunda* and *P. fuscus*, the biggest increase of volume secretory part of thyroid gland per gram of body weight occur between stages XVII and XXI, which corresponds to the transition period between the pro- and metamorphosis. In *R. esculenta* and *B. calamita* it is highest between stages II and VI, whereas in *B. viridis* and *B. bufo* between stages X and XVII prometamorphosis.

The total area of thyroid follicles per gram of body weight, in four of the eight investigated species, i.e. *R. bedriagae, R. lessonae, P. fuscus* and *B. calamita* are the highest increase of parameters followed between stages X and XVII, in *R. esculenta* between stages II and VI, in R. ridibunda between stages VI and X.

These studies show that the development of secretory part of thyroid gland (volume and area of secretory part per gram of body weight) have different course in tadpoles of different species of Anura.

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The embryonic development of the river lamprey *Lampetra fluviatilis* L. in controlled conditions

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Evolutionary, lampreys are the oldest group of aquatic vertebrates (Gess, 2006), distinguished by the absence of jaws and therefore classified as belonging to the superclass agnathans (Agnatha). The oral aperture of lampreys is a sucking disc with conical teeth, and the shape, position and number of teeth is a taxonomic feature (Renaud, 2011). Lampreys are characterised by a very interesting embryonic development (Rodriguez et al., 2001), which, in the following paper, has been described using the river lamprey *Lampetra fluviatilis* as an example species.

The spawn of river lamprey originated from spawners caught in May in the Vistula Lagoon. The sex products were obtained in controlled conditions, with no hormonal stimulation. The embryonic development proceeded in water of the temperature of 12°C, with egg cells placed in Weiss mini-jars, set in a hatching system (Kujawa et al., 2000). A fertilised ovum is 1.0–1.3 mm in diameter. After about 9 hours, the cell divides symmetrically into two parts. The subsequent cell cleavage was observed at 14 h of development. At 19 h the cell divides into 8 parts. The blastopore closes at day 7 of the development. At that time, it was possible to distinguish the contours of the cephalic region and notochord. The cephalic region adheres closely to the yolk sac. The contours of the cephalic region and the notochord become more visible at 8 d of the development. At day 9, the anterior part of the embryo elongates. Slow movements of the embryo become observable. As the embryo grows, its movements intensify and the anterior and posterior parts are distinctly distinguished. Shortly before hatching, embryos make energetic movements inside the eggs. Hatching begins at day 20 of the development and proceeds for 2–3 days. After hatching, river lamprey larvae are 4.7 to 5.1 mm long.

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Measurments of the parts of aorta in human embryos

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The early development of the aorta occurs with rapid changes in blood flow and function of the heart which provides changes in vascular development and growth. Measurements which were taken between the internal diameters of ascending aorta (1 to 2 cm beyond the valve), and descending aorta (1cm distal to the insertion of the ductus arteriosus). Hilde van Meurs-van Woezik (1988) found that the ratio of internal diameters of ascending part of the aorta were greater than in the descending part of the aorta.

From data presented by Ursell (1991) concerning the diameters of developing blood vessels it was shown that aortic isthmus was smaller than the aortic valves or descending part of the aorta.

The aim of our study was to compare the length and width of ascending part of the aorta, arch and descending part of the aorta in human embryos at stages 18–23 (44–56 days).

Study was performed on 16 human embryos at stages 18–23 from the Collection of Department of Anatomy, University of Medical Sciences in Poznań. Age of embryos was determined according to 23 international Carnegie stages and was expressed in postfertilizational days. Embryos were embedded in toto and sectioned in sagittal, frontal and horizontal planes. Serial sections were stained with various histological methods and impregnated with protargol (Bodian method) and silver gelatin using the method of Pearson and O'Neil. Parts of the aorta were measured with Axio Vision LE computer program.

In the 7th week (stage 18, 44 days) the length of the arch of aorta was taken from the aortic valve to the orifice of aortic duct and arises the length 0,73 mm at this stage. The width of ascending aorta was between (0,24–0,49 mm) and for descending aorta (0,24–0,44 mm). The length of ascending aorta was 0,68 mm. The length of arch of the aorta elongates to 1,29 mm. The length of descending aorta was 1,44 mm. The width of aortic ostium was 0,39 mm.

In stage 20 (49 days) width of arch of the aorta arises 0.58 mm.

In 8th week (stages 21–23, 51–56 days) the width of arch of the aorta remain the same, the progress in elongation was observed in particular parts of the aorta. At the end of embryonic period the width of the arch of the aorta arises 0.64 mm. The width of ascending aorta increases to 0,61 mm and in the descending aorta was this same.

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Arabinogalactan proteins from gum arabic enhance haploid plant regeneration in barley anther culture

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Androgenesis is a process were microspores are switched from gametophytic to sporophytic pathway giving rise to embryos regenerated into plants. The usefulness of androgenesis in basic research and plant breeding is well known. High effectiveness measured by number of green plants regenerated is a prerequisite for application of androgenesis in these studies. Arabinogalactan proteins (AGPs) are a hydroksyproline-rich proteoglycans, which are involved in plant growth and development including cell proliferation, expansion, differentiation, programmed cell death and embryogenesis (Majewska-Sawka and Nothnagel, 2000). Addition of different AGPs to the media was shown to increase the production of green plants from wheat microspores (Letarte et al., 2006).

The objective of this study was to investigate the effect of commercially available source of AGPs – gum arabic (GA) on the course of androgenesis in barley (*Hordeum vulgare* L.) anther culture.

We showed that addition of 5 and 10 mg/L GA to induction medium resulted in about 1.6 and 2.3-fold higher frequency of plant regeneration respectively, when compared with cultures without GA. To confirm the role of AGPs in androgenic process we used β-D-glucosyl-Yariv reagent which is considered as AGPs inhibitor (Trifunović et al., 2014). Addition of 30 μM Yariv to the medium with 10 mg/L GA reduced the frequency of plant regeneration about 5 times indicating a key role of AGPs in androgenic regeneration from barley anthers.

Histological observations revealed that first androgenic division was asymmetrical and occurred after 1 or 2 weeks on induction medium with or without GA. Further divisions of newly formed cells were similar on both types of media and resulted in multicellular structures which gave rise into callus from which embryoids were developed. Several morphological abnormalities of developing embryos were observed and they were more pronounced on media lacking GA. The process was markedly accelerated on media with GA.

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Changes in the structure of *Drosera spatulata* Labill. cells as response on various spectral composition of radiation in *in vitro* cultures

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Under environmental conditions, plants are exposed to a number of biotic and abiotic stress factors. One of the major abiotic stress is light stress which results from changes in the intensity and spectral composition of radiation. Influences on the response system to stress factor allows to acclimatize the organism by modifying the metabolism and ways of development (Knight and Knight, 2001). *Drosera spatulata* Labill. (sundew), a carnivorous plant has the ability to produce secondary metabolites from naphthoquinones group, important in the pharmaceutical field (Banasiuk et al., 2012). Due to overgrow of natural habitats of sundews by other plants, *D. spatulata* Labill. may be exposed to the limited access to light.

The aim of the study was to observe the changes at the cellular level of sundew, causes by light stress.

Root explants were placed on 1/2 MS medium with no growth regulators. Tissue cultures were treated by: LED lamp (maximum emission in the blue range 442 [nm] and 634 [nm] in red range, pink colour), darkness and fluorescence lamp. The intensity of lights was 120 [μmol(quantum) × m^{-2} s^{-1}], photoperiod 16/8 (light/dark), temperature 24 ± 1°C. To illustrate and analyze cells changes, light and electron microscopy techniques were used.

The application of high radiation intensity of both lights resulted in the formation of an extracellular matrix. These structures differed depending on the spectral composition of used radiation. The various light conditions (darkness and both lamps) caused various direction cells development.

The observed structural changes of extracellular matrix arising in connection with the high-intensity light suggest their protective role in the cells differentiating. Furthermore, experiment has shown that not only the quantity but also quality of radiation can modify the construction of such structures. This study gives the foundation for further work responding to questions about the importance of extracellular matrix in the process of acclimation of cells and plants organogenesis.

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Histogenesis of the thyroid gland of the domestic cat during prenatal development; preliminary studies

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The thyroid gland as the first endocrine gland originates from the fifth pharyngeal pouch and the ventral epithelial thickening in the pharyngeal portion of the foregut. The thyroid hormones are necessary for regulating the basal metabolic rate, somatic growth, psychic growth, calcium metabolism and circadian rhythm in fetus, neonate and adult stages. Therefore, it is important to examine the histogenesis of thyroid glands to describe the particular stages of development.

Cat fetuses from ca. 28, 40, 50 and 63 day p.c. were preserved in 10 % formalin and dissected for description of thyroid gland position. The samples of thyroids was processes routinely for LM observation. Paraffin sections were stained with trichrome Masson-Goldner, PAS, PAS AB staining).

The results allowed us to describe a main stages of prenatal development. The first undifferentiated stage of thyroid primordium last up 30 day day of p.c. On crossections of thyroid the bands of cells with rich, loose network of capillaries were founded.

In fetuses from 40 day starts the formation of follicles with cuboidal epithelium was noted. The follicles were predominantly founded in outer, peripheral area of thyroid. Under capsule of thyroid built from onelayered flat epithelium, the clusters of capillaries were observed.

After 50 day the number of follicles positioned also in the middle part of thyroid grows rapidly. The thin connective tissue septa between follicles have a rich network of blood vessels. On crossections of thyroid between 55–61 day of p.c. colloid were seen in lumen of follicles.

Effect of kwercetin and caffeic acid on maturation of ABA-treated somatic embryos of tulip 'Apeldoorn'

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ABA enhance an embryo development and quality (Seo and Koshiba, 2002; Maślanka and Bach, 2005), whereas phenolic acids and flavonoids probably have been implicated in developmental processes such as cell division (Bagni and Tassoni, 2001; Kobylińska and Janas, 2015).To optimize development of somatic embryos of tulip 'Apeldoorn', torpedo stage embryos, 5–10 mm length, were placed on pre-maturation MS medium containing 5 M picloram, 1 M 6-benzylaminopurine (BAP) and 10 μM abscisic acid (ABA) for one week. Then, the embryos were transferred on maturation MS medium containing 2.5 μM BAP, 0.25 μM 1-naphthaleneacetic acid (NAA) (control) and treated with 1478 μM (0.05%) kwercetin or 555 μM (100mg/l) caffeic acid (CA) for ten weeks. During the first week of the experiment the cultures were maintained in darkness and then also under light conditions.

The obtained results have revealed that both kwercetin and CA had no significant impact on fresh weight, dry weight and length of the somatic embryos in comparison with control. The greatest percentage of leaf-forming embryos (non dormant) was observed after CA treatment in darkness. Considering light conditions, it was observed that the embryos under light increased their fresh weight and length in comparison with embryos from darkness. It was caused by higher absorption of water under light conditions, as indicated by lowering dry weight. During maturation, all of the embryos became malformed in similar level. Light also adversely affect the embryos malformations.

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The structure and ultrastructure of the egg capsules of *Isoperla* species (Insecta, Plecoptera: Perlodidae)

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The aim of the investigations was to describe the structure and ultrastructure of the egg capsules of five Plecoptera species belonging to the genus *Isoperla: I. grammatica, I. sudetica, I. tripartita, I. pesici* and *I. goertzi* inhabiting different environments in several geographical regions of Europe. The microscopy analyses have shown that the egg capsules of investigated species are composed of a vitelline envelope, chorion and extrachorion. The vitelline envelope is thin and externally placed to the oolemma. The chorion is situated next to the vitelline envelope and usually thick (except of *I. sudetica*). In the chorion numerous aeropylar canals which are responsible for gas exchange occur. The number and arrangement of those aeropylar canals is different in various Isoperla species. Scanning electron microscopy analyses have indicated that there are differences on eggs surface between analyzed species. In four of investigated *Isoperla* species (except of *I. pesici*) on the posterior region of egg capsule attachment structure (disc) with umbrella-like structures occurs. The lack of attachment disc in *I. pesici* is compensated by highly developed extrachorion surface.

The results of the study, when comparing the different species, showed that the egg capsules of closely related stoneflies, belonging to the same genus *Isoperla*, are constructed according to common general scheme, but are species-specific and morphologically adapted to the particular habitat, where the development takes place.

Ultrastructure, distribution and transovarial transmission of symbiotic microorganisms in the mealybug, *Coccura comari* (Insecta, Hemiptera: Pseudococcidae)

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In the body of scale insects and other hemipterans feeding on plant sap commonly occur symbiotic microorganisms. It is believed that these symbionts are responsible for the synthesis of amino acids missing in the diet of their host insects. The histological and ultrastructural observations have shown that in the body of *Coccura comari* numerous large pleomorphic bacteria are present. These symbionts are harbored in the cytoplasm of specific cells of mesodermal origin termed bacteriocytes. The bacteriocytes form large paired organs termed bacteriomes which are localized in the close neighborhood of ovaries. The results of molecular analysis based on 16S rRNA gene sequences of endosymbionts have shown that the bacteria colonizing the body of *C. comari* belong to the genus Tremblaya (β-proteobacteria). Symbiotic bacteria are transmitted from one generation to the next transovarially. The ovaries of *C. comari* are composed of numerous short telotrophic ovarioles which are subdivided into two parts: an anterior trophic chamber (tropharium) and posterior vitellarium. In the vitellarium a single oocyte develops. The developing oocyte is surrounded by a single layer of follicular cells and is connected with the tropharium by means of broad nutritive cord. The bacteria infect follicular cells surrounding the anterior pole of the vitellogenic oocyte. Ultrastructural observations have revealed that symbiotic microorganisms migrate through the cytoplasm of follicular cells. Then, the bacteria accumulate in the perivitelline space.

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Embryo sac development and the problem of low seed set in alfalfa (*Medicago sativa* L.)

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Low seed set in *Medicago sativa* L. is the main reason of the slow selection response which is a serious problem in alfalfa breeding. Alfalfa inflorescence mutants used in breeding programmes could increase the seed yield, and improve the selection response. In our former and recent studies, we use the long peduncle (*lp*), branched raceme (*br*) and top flowering (*tf*) inflorescence mutants, all presenting increased flower numbers per inflorescence. We have shown that the poor fertilization effectiveness as well as young seed gradual degeneration resulted in poor seed set in those mutants of *M. sativa* (Mól et al., 2011). Moreover, callose deposition in the ovules before fertilization and in the young seeds seems to affect the ovule or seed development (Mól et al., 2014). Now we show the preliminary results on the embryo sac formation in the alfalfa *lp*, *br*, and *tf* mutants. Embryo sac development appeared to be of the Polygonum-type, however more precise analyses on megaspore formation are necessary. In mature embryo sacs the elongated and vacuolated egg cell, two small synergids, and the central cell (with two polar nuclei or the secondary nucleus) were observed. Numerous starch grains were usually found in the central cell. The antipodal cells were often hard to detect by clearing method due to presence of a hypostasis-like structure at the chalazal end. Ovaries contained 8–11 ovules, and just before flower opening 75% (54–91%) of ovules contained normally formed embryo sacs. Approx. 24% (9–45%) of embryo sacs were deformed. Among malformed embryo sacs narrow ones with no visible cells were found. Occasionally short embryo sacs with the small egg apparatus and missing elongated chalazal part were seen. In conclusion, disturbances in the embryo sac development participate in poor seed setting in alfalfa inflorescence mutants. However the frequencies of deformed gametophytes are too low to be the only explanation of very low seed set in this species.

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Reproductive system in *Dactylorhiza incarnata* var. *incarnata* (L.) Soó

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Dactylorhiza incarnata belongs to diploid taxa (2n=40) within *Dactylorhiza*, where unidirectional hybridization between the same diploid parental lineages led to allotetraploid complex establishment (Nordström and Hedrén, 2009). Molecular techniques allow determine the degree of relatedness between the tetraploid offspring and parents (Balo et al., 2016). However, when and in which way the genome is duplicated (due to abnormalities in parental gametes formation; after fertilization – during zygote division or as a result of disturbances in offspring meiosis), it remains a challenge for embryologists. We investigated the development of ovules for different strategies of reproduction: open pollination, induced autogamy and allogamy, and putative apomixis. Analysis of sectioned or cleared material showed that in flower bud, ovules were poorly developed (at archespore or meiocyte stage), while pollinia contained 1- or 2-nucleate pollen grains. Megasporogenesis was monosporic and finished in triad of megaspores, of which chalazal one became functional. A nondysjuction during first meiotic division we observed in a few ovules. Formation of mature, Polygonum-type female gametophyte occurred 5–7 days after induced self- or cross-pollination; embryogenesis started 2-3 days later. Open pollination and apomixis were unsuccessful, as only degenerated ovules we noted 9–10 days after anthesis. Development of female gametophytes until maturity took place in emasculated and unpollinated flowers, despite the lack of pollination trigger. We suppose self-pollination in *D. incarnata* is unlikely even though there are no of prezygotic barriers. Whether the incidental disturbances during megasporogenesis might be important for hybridization and polyploidization remain to be verified.

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Localization of poly(A) RNA in male gametes of *Arabidopsis thaliana* and *Hyacinthus orientalis*

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The mature pollen grain of flowering plants represents a unique structure with a key function in sexual plant reproduction. Depending on the timing of generative cell mitosis, two sperm cells may be formed during pollen development in the anther (tricellular pollen grain, *Arabidopsis thaliana*) or during pollen tube growth (bicellular pollen grains, *Hyacinthus orientalis*). Pollen tube formed by a vegetative cell transports of gametes through the pistil tissue to the embryo sac where double fertilization takes place.

In the recent years, the genome-wide analysis of the male gametophyte transcriptome in *A. thaliana* suggest that sperm cells possess a transcriptome with the unique composition also with sperm cell-specific transcripts. The aim of our study was to determine by FISH (fluorescence in situ hybridization) the spatial and temporal localization of poly(A) RNA in male gametophyte cells of *A. thaliana* and *H. orientalis* with particular account of sperm cells. In the mature pollen grain of *A. thaliana* sperm cells accumulate a pool of polyadenylated RNA, which is stored until may be translated after rehydratation and during pollen tube growth. In the pollen tube the high accumulation of poly(A) RNA in the cytoplasm was still localized. In hyacinth, after sperm cells formation poly(A) RNA in both of gametes was visibled in the form of numerous granules. The described changes in the distribution of poly(A) RNA in sperm cells of both species may reflect the metabolic activity of cells and also may indicate their at least partial metabolic autonomy from the pollen tube. We also postulate that sperm cells transfer paternal transcripts during fertilization (we know that the egg cell of hyacinth does not accumulate large amounts poly(A) RNA and fertilization induces the chromatin remodeling and the activation of the zygote and endosperm genome). The research was supported by the National Science Centre (NCN) grant 2011/03/D/NZ3/00603

The analysis of the development of the female gametophyte of *Lycopersicon esculentum* Mill., *Solanum melongena* L. and *Solanum sisymbriifolium* Lam. isolated from the selected developmental stages of the flower buds

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In plant breeding, indication of optimal pistil stage for pollination, lead to increasing the frequency of the embryos and seeds formation after selfing or interspecific/intergeneric crosses. During evaluation both, stigmas and embryo sacs receptivity (usually correlating) should be considered. For the purpose to specify the best stage of pistils for pollination we have assigned the successive patterns of embryo sac development with a series of defined floral developmental stages of 3 representatives of Solanaceae family – *L. esculentum*, *S. melongena* and *S. sisymbriifolium*.

According to the flowering period we have prepared a chronogram comprised of 4 phenophases of the flower bud development: closed flower bud (days before anthesis D3, D2, D1) and opened flower (day of anthesis DA). To standardize the developmental stages of the sampled flower buds we randomly selected 3 different plants of each species. For the embryological analysis, ovules were isolated from pistils of the specified flower buds, fixed in FAA solution (90 ml of 70% ethanol + 5 ml of formalin + 5 ml of glacial acetic acid), embedded in paraplast, sectioned with a microtome (Reichert, section thickness – 12 μm), stained with iron haematoxylin (3 g/500 ml of 80% ethanol) and counterstained with fast green FCF (0.3 g/100 ml of clove oil). Stained permanent slides were enclosed in Entellan (Merck). Ovules with embryo sacs were analyzed under the light microscope (Axioscope A.1. Zeiss). The micrographs were captured using Axiovision 8.1 Software.

Embryological analysis allowed to specify the best stage (stages) of pistils for pollination for all tested species. The differences between the examined species in the time of the embryo sacs maturity was significant. In *L. esculentum* and *S. sisymbriifolium* well developed embryo sacs comprised of egg apparatus as secondary cell nucleus were present in closed flower buds one day before anthesis (D1) and in the day of anthesis (DA). Additionally, at this period a frequent germination of pollen grains after self-pollination were observed. In *S. melongena* well developed embryo sacs were visible in ovules isolated from flower buds already 2 days before anthesis (D2), however pollen grains germinated on stigmas not as frequent as in the later stages (D1, DA). The structure of embryo sacs at the D1 stage in all tested species show their receptivity and suggest, that it is the best stage for pollination. More details concerning the structure of embryo sacs in examined developmental stages will be demonstrated on the poster.

Indole metabolism in the embryonic pineal gland of the domestic turkey – a preliminary study

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The main pineal indolic hormone, melatonin (MLT), synthesized in the diurnal and seasonal rhythms, has been widely examined in almost all vertebrate species. However, the knowledge about the entire indole metabolism including all precursors and metabolites of MLT biosynthesis pathway is scarce. The studies on indole metabolism in birds were carried out exclusively on the duck and goose pineal glands. In the avian embryonic life only MLT secretion was examined, mostly in chick embryos. In the present study we characterized for the first time the changes in contents of nine indoles occurring during embryonic development of the turkey pineal gland.

The study was performed on 14-, 16-, 18-, 20-, 22-, 24- and 26-day-old turkey embryos (assigned below as 14ED – 26ED) incubated under 12L:12D cycle. The embryos were killed at 12.00 and 24.00, the pineal glands were immediately removed and frozen at -75°C. The indole contents were measured using HPLC with fluorescence detection.

The content of tryptophan (TRP) – a precursor of indole synthesis – was at high level in all examined embryos and showed no significant changes during development. The level of 5-hydroxytryptophan (5-HTRP) increased stepwise up to 22ED and then remained constant. The serotonin (5-HT) level raised between 22ED and 26ED. The content of N-acetyl-serotonin (NAS) remained at low level up to 18ED, then markedly increased, and from 22ED showed day-night variations. MLT content was lower than NAS content up to 24ED, then rapidly increased. It revealed diurnal changes at 22ED, 24ED and 26ED. The contents of 5-hydroxytryptophol (5-HTOL) and 5-hydroxyindole acetic acid (5-HIAA) stepwise increased from 16ED and 18ED, respectively. 5-methoxytryptophol (5-MTOL) was not detectable in the pineal gland during the whole period of embryonic development. 5-methoxyindole acetic acid (5-MIAA) occurred at measurable levels from 24ED, and from this time-point showed diurnal changes with low levels at night and high levels during the day.

Summing up, the contents of only three indoles (NAS, MLT and 5-MIAA) show the diurnal fluctuations at the end of embryonic life. The quantitative relations between NAS and MLT suggest that the activity of N-acetyl-serotonin O-methyltransferase (ASMT), but not the availability of NAS, determines the level of MLT synthesis in pineal glands of turkey embryos. The occurrence of 5-MIAA at low levels and the lack of detectable amounts of 5-MTOL, despite the presence of considerable amounts of their precursors, also point to the rate-limiting role of ASMT in the indole metabolism.

Searching for sources of apomixis – reproduction in *Boechera stricta*

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Boechera stricta (Graham) Al-Shehbaz has been shown to be predominantly diploid and sexual, in contrast to other *Boechera* species that are facultative apomicts, and highly variable with respect to ploidy, morphology, and genetic polymorphism (Böcher, 1951; Voigt-Zielinski et al., 2012). *B. stricta* seems well studied and morphologically distinctive, that their genetic contribution to any apomictic hybrid is immediately apparent (Windham et al., 2016). Very few notes reporting the cases of apomictic development in *B. stricta* (Aliyu et al., 2010) may, however, indicate this species is not so strictly sexual, as it seems to be. Step by step investigation of ovule, gametophyte, embryo and endosperm development we conducted in two *B. stricta* accession lines, by using transmission electron microscopy, clearing method and Nomarski and epifluorescence microscopy. Beyond typical sexual path, we observed: diversity in ovule development, Taraxacum-like type of dyads formation during female sporogenesis, disturbances in male gametogenesis, and what's more, embryo abortion and parthenogenesis. We propose to consider *B. stricta* as sexual species, that has the capability to reproduce diplosporously. These findings will be important for future investigations, especially to understand epigenetic mechanism of apomixis.

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Sexual reproductive traits in tetraploid *Pilosella officinarum* (Asteraceae, Cichorioideae): DIC microscope study of cleared whole-mount tissue

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Hieracium species are highly polymorphic which results from facultative apomixis, frequent interspecific hybridization, and polyploidy. In Europe, this genus is divided into the subgenera *Hieracium* s.str. and *Pilosella*, which are now considered as two separate genera. Both *Hieracium* and *Pilosella* comprise sexual and apomictic species but differ in the type of gametophytic apomixis. Diplosporous female gametophyte formation occurs in *Hieracium* s.str. species, while in *Pilosella* species apomixis is aposporous.

Pilosella officinarum F. Schultz & Schultz-Bip. (syn. *Hieracium pilosella* L.) represents the genus *Pilosella* and consists of several ploidy cytotypes, ranging from diploid to decaploid, that exhibit different reproductive modes (Gadella, 1987). Three ploidy levels are common in Europe: tetraploids (mostly sexual), pentaploids (mostly apomictic, rarely with sexual individuals), hexaploids (both sexual and apomictic) (Kahulec and Krahulcová, 2011).

This study focused on the embryological processes occurring in the ovules of tetraploid *P. officinarum* (2n=4x=36) from the southern Poland. A clearing tissue technique with methyl salicylate has been applied to the examination of non-stained, whole-mount young ovaries, dissected older ovules or mature embryo sacs with the use of differential interference contrast microscopy. Cyto-embryological analysis showed that the reproduction of the investigated specimens involves a regular monosporic megasporogenesis, female gametophyte formation of the *Polygonum* type, and embryo and endosperm development after fertilization of the egg and central cells. The division of zygote and early embryogenesis are preceded by the formation of cellular endosperm.

Thus, the results obtained in this study are in accordance with previous reports (Pogan and Wcisło, 1995) and confirm that tetraploid *P. officinarum* reproduces sexually.

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Evolution of lepidosaurian developmental sequences

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Besides turtles and archosaurs, Lepidosauria are one of the three major clades of extant reptiles. This group includes over 9,000 species of squamates (lizards and snakes) and one species of tuatara. Evolutionary history of lepidosaurs dates back to the beginning of the Mesozoic, about 240 million years ago. While the division of lepidosaurs into rhynchocephalians (tuatara and kin) and squamates is accepted, the relationships within the latter clade are a matter of debate because of fundamental differences between phylogenetic hypotheses based on morphology and molecular data (e.g. Losos et al., 2012). Data derived from developmental sequence, i.e. order in which certain structures develop, may contain phylogenetic signal, yet they were surprisingly rarely used in squamate phylogenetics (with Werneburg and Sánchez-Villagra, 2015 being one exception). Here, we adopted data on embryonic developmental sequences of 21 species of lizards and snakes from Andrews et al. (2013), supplemented them with developmental sequence of the tuatara (*Sphenodon punctatus*) and analysed in a phylogenetic context. Cladistic analyses conducted using these developmental sequences do not support strongly either morphological or molecular phylogeny. However, in most of these analyses, a majority of iguanian species form a group near the base of the cladogram, which reminds the morphological topology. Event-paired data are equally consistent with either morphological or molecular hypothesis but the continuous dataset provides minimally shorter tree for the former (144 steps vs. 146 steps). This means that the morphological topology is slightly better explained by these characters.

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The studies of crustacean embryo development – freshwater shrimp *Neocaridina heteropoda*

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Embryogenesis is an important stage in animal development, because during this stage the embryo's body undergoes significant transformation which can prepare the entire organism for proper functioning in adult life. Despite the fact, that research studies on embryogenesis and postembryonic development are very interesting, they are very difficult to conduct. Therefore, a carefully chosen set of non-destructive microscopy techniques has to be implemented in such studies in order to maximize the imaging results without complex sample preparation procedures.

N. heteropoda is an important species for comparative developmental study for other crustaceans, because it is easy to breed and easy to possess. Furthermore our result can be easily related to other crustacean species, mainly malacostracan.

Our study helps to obtain the new knowledge about processes which undergo during embryonic development. The techniques of visualization that we used during our studies, help us to obtain three dimensional (3D) datasets on those samples. It is not possible when use either traditional light or electron microscopy. The new information can give us much more precise understanding of body plan development and the processes of morphological development.

Short-term pollen storage of pollination strategy in inter-generic hybridization of *Salix* × *Populus*

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Fast growing species of *Salix* and Populus genera are important plant crops cultivate as renewable feedstock for bioenergy in Europe. Plant growth potential and high biomass yield production in these species are the important characteristic of cultivated clones and these factors are base of plant selection. Broadening the genetic diversity in Salix genus with hybridization technique increase the possibility of creation new plants for selection purpose. Our earlier studies demonstrated the possibility that inter-generic hybrid embryos of *Salix* × *Populus* could be obtained by application of *in vitro* fertilization and *in vitro* embryo rescue techniques. For more successful inter-generic hybrid breeding of the species an efficient pollination control system is necessary to be established. The experimental pollination difficulty of distant crosses is conducted with synchronization of flowering of female and male components to apply only fresh pollen to the receptive stigmatic surfaces. The aim of the study was to develop effective pollination method for intergeneric hybridization of *Salix* × *Populus* with short-term storage pollen. Crosses were performed under cultivation room conditions (20±1°C, humidity 45%, light intensity of 250–300 $\mu molm^{-2}s^{-1}$).

To break flower bud dormancy, branches of *S. viminalis* were kept in the growth chamber where pollination was performed. Branches of *Populus spp.* were kept in separated laboratory rooms. At time of anthesis, pollen was shacked out from *Populus* catkins over Petri dishes and the part of collected pollen was storage at 4°C. Fresh and store pollen were transferred onto receptive stigmas of two clones of *S. viminalis*. Pollen viability of *P. tremula* was examined by stigma germination test at 24 and 48 hrs after cross pollination. The viability of pollen was assessed in terms of percentage of germination. The data observed were submitted to statistical analysis (Duncan's Multiple Range test).

Experimental results indicated that fresh and stored pollen of *P. tremula* were germinated on *S. viminalis* stigmas. Pollen stored at +4°C showed reasonable germination on stigmas up to 2 weeks but later on the germination decreased from 56% to 32%. The observations of germination and tube growth of stored pollen indicated no deviations from the fresh pollen. Stored pollen grains germinated and pollen tube penetrated stigma and style and growth into ovary with the comparably range to fresh pollen. There were no significant differences in pollen germination on stigmas between *S. viminalis* clones. The conditions of growth chamber turned to be optimal for *in vivo* germination and tube growth. The indicated possibility of *Populus* pollen storage for two weeks increases the chances in hybridizing success in distant pollination of *Salix* and subsequently in hybrid embryo formation.

Effects of nitrates on early ontogenetic development of *Labeotropheus trewavasae* (Teleostei: Cichlidae)

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Nitrogen compounds such as nitrites, nitrates and ammonia are widespread in the environment. Their number is increasing with the progress of urbanization leads to a well-known phenomenon of the lakes – eutrophication. The effect of ammonia and nitrite on living organisms is well described in the literature, however, less information exist on nitrates and their negative impact on the development of fish fauna. The aim of this study was to determine the effect of nitrate at a concentration of 300 mg/l on the species of the cichlid fish, *Labeotropheus trewavasae*. This species inhabits the Lake Malawi (Africa), where there is a real risk of eutrophication. Research hypothesis assumed that incubation of early developmental stages (embryos and larvae) *L. trewavasae* in nitrate concentration of 300 mg/l lead to negative changes in their ontogenetic development. The research was conducted under laboratory conditions, where water parameters was stable such as the nitrate concentration, temperature, pH and oxygen content.

The embryonic and larval development *L. trewavasae* was investigated. Biometric measurements were made using a microscope and professional software. The results shows, that exposure of fish to the nitrate in a concentration of 300 mg/l have a significant effect on their development. During embryonic development is observed slower body growth of the embryo and the slower rate of resorption of the yolk, used to nourish the embryo. Consequently, after hatching the larvae *L. trewavasae* are smaller. Additionally disorder are the proportions of the body of the newly-hatched larvae. Larval period is extended, which is caused by slow absorption of the yolk sac. During this period, the disproportion between the different parts of the body is further deepened.

Pelargonium zonale var. "Kleiner Liebling" as a model plant for haploid plant studies

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Pelargonium spp. is one of the most popular garden plants in the world, it has a considerable economic importance in the ornamental plant market. However, some of species from this genus are used in the pharmacy, aromatherapy, perfumery and cosmetic industry as well. Most of approximately 280 species of the genus *Pelargonium* (fam. Geraniaceae) are native for South Africa. *Pelargonium zonale* var. 'Kleiner Liebling' is one of the pelargonium dwarf varieties with chromosomes number nine, and it is true haploid (monoploid) (Daker 1966), which makes it the interesting model plant. Although the abnormalities in the reproductive of polyploid plants are common, this is the first report of the preliminary reproductive aspects among the *P. zonale* haploid and diploid cytotypes.

The aim of this study was to conduct a comparative analysis of reproductive processes of haploid and diploid cytotypes of *P. zonale* var. "Kleiner Liebling". The histological analysis confirmed the presence of ovules in pentalocular ovaries in diploids and haploids and additionally the tetralocular in some haploid individuals. Both cytotypes formed two types of ovules: campylotropous located in upper position and anatropous down of the ovary chamber in the longitudinally sections. In closed, young buds we observed the presence of stamens, which degenerated earlier in haploids than in diploids individuals. In closed, young buds we observed the presence of stamens, which degenerated earlier in haploids than in diploids individuals. The histological sections of microsporangia revealed the absence of archesporial tissue, and the poor structure of anther wall. Anther wall in haploids was a single layer, formed by epidermis, and anther wall in diploids consisted of two layer composed of epidermis and middleyer.

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Chromosome bouquet, Balbiani body and the polarity of *Thermobia domestica* oocyte

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The meiotic division which guarantees maintenance of a genetic diversity consists of several stages, with prophase I being the longest and the most complex. We decided to follow the course of initial stages of meiotic division in ovaries of an apterygotous insect, *Thermobia domestica*. We show that germaria of this species contain numerous germline cells that can be classified into three main categories: cystoblasts, meiotic oocytes and growing previtellogenic oocytes. The cystoblasts are located most apically. The meiotic oocytes occupy the middle part of the germarium, and the previtellogenic oocytes can be found in the most basal part near the vitellarium. Analyses of the semithin sections and squash preparations have shown that zygotene – pachytene meiotic chromosomes rearrange and form the so-called bouquet. The telomeres of the bouquet chromosomes are attached to a relatively small area (segment) of the nuclear envelope. Next to this envelope segment the nucleolar organizer is also located. We show that in concert to sequential changes inside the oocyte nuclei, rearrangement of organelles within the ooplasm (oocyte cytoplasm) takes place. It should be stressed however, that these early polarities of oocytes (nuclear and cytoplasmic) of *Thermobia* are transient. During diplotene, the chromosomes are distributed more or less uniformly, and the Balbiani bodies start to disperse in the ooplasm. Finally, our observations have indicated the presence of lamp-brush chromosomes in previtellogenic oocytes. In the close vicinity to the lamp-brush chromosomes characteristic spherical nuclear bodies are present.

Morphological, histological and ultrastructural features of *B. nymphopolitanum* Kraenzl flowers (*B.* section *Lepidorhiza* Schltr., Bulbophyllinae Schltr., Orchidaceae)

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The genus *Bulbophyllum* consists of pantropical, fly-pollinated orchids (myiophilous or sapromyiophilous). Sapromyiophilous flowers imitate brood and food sites of flies (Diptera) using visual and olfactory effects to attract them as pollinators. The flowers of *Bulbophyllum nymphopolitanum* fulfil features that characterize sapromyophilous flowers. The aim of this work was to provide micromorphological, histochemical and ultrastructural studies of flowers of *B. nymphopolitanum* and determine the occurrence of nectaries and osmophores. *B. nymphopolitanum* had the nectary in longitudinal groove in the base of the lip and the osmophores were located in prolonged apices of sepals. TEM studies revealed that the cuticle in both species was composed of two layers: a reticulate cuticle layer (with microchannels) and amorphous cuticle proper. Through microchannels the exudation is transported to the surface of osmophores and nectaries. Plastids in lip, collumn and lateral sepals were starchless, which could be caused by their hydrolization during anthesis. Profusion of plastoglobuli in plastids, where the synthesis of fragrance components may occur, as well as numerous lipid bodies, indicated that volatile production took place.

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Mitochondrial activity in hepatopancreas of *Neocaridina heteropoda* (Crustacea, Malacostraca) exposed to starvation and re-feeding

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The aim of our experiment is to study changes in mitochondrial activity of the digestive system in freshwater shrimp *Neocaridina heteropoda* (Crustacea, Malacostraca) exposed to starvation and re-feeding. Studied species is a freshwater, omnivorous, 2–3 cm in length shrimp from Taiwan. Experiment was conducted on adult specimens (males and females) that have been bred in laboratory shrimp tank at constant conditions, i.e. T=24°C, pH=7 and GH=9od. Adult animals were held in separate containers with the same parameters as in the main culture. Shrimps that have been starved 7, 14 and 21 days were fed again for the next 4, 7 and 14 days. Changes of mitochondrial activity have been observed by flow cytometry and confocal microscopy. Changes in mitochondrial transmembrane potential have been monitored with using the JC-1 cationic dye which accumulation in mitochondria depends on the magnitude of mitochondrial potential. The JC-1 dye differentiates cells with a high (red fluorescence) and low mitochondrial potential (green fluorescence). The mitochondrial potential has been detected according to the progress of starvation: the decreased amount of mitochondria exhibiting high membrane potential has been observed. It was also found, that the regeneration, which occurs after refeeding period can be correlated with the increasing amount of mitochondria with high membrane potential.