ACTA BIOLOGICA CRACOVIENSIA

SERIES BOTANICA

Vol. 54 suppl. 1 2012

ABSTRACTS

XXX Conference on Embryology
Plants • Animals • Humans

May 16–18, 2012
Jurata, Poland

Polish Academy of Sciences – Cracow Branch
Jagiellonian University
Polish Academy of Arts and Sciences

ACTA BIOLOGICA CRACOVIENSIA
Series Botanica

The Official Publication of the Biological Commission of the Polish Academy of Sciences – Cracow Branch and the Jagiellonian University

DEVOTED TO PLANT ANATOMY, MORPHOLOGY, CYTOLOGY, GENETICS, KARYOLOGY, EMBRYOLOGY, TISSUE CULTURE, PHYSIOLOGY, BIOSYSTEMATICS, PHYTOCHEMISTRY, SECONDARY METABOLISM, PHARMACOLOGY, BIOACTIVITY OF PLANT NATURAL PRODUCTS, BIOTECHNOLOGY, MOLECULAR PHYLOGENETICS AND PHYLOGEOGRAPHY

ESTABLISHED 1958

The edition of this supplement is financed by the Polish Academy of Arts and Sciences.

EDITORIAL NOTE
The abstracts have been printed as received, and no proofreading or corrections have been made. Thus, the contents of the abstracts are entirely the responsibility of the contributors. In the Index, the names of authors (in alphabetical order) are accompanied by the respective page numbers.

ACTA BIOLOGICA CRACOVIENSIA Series Botanica is published twice a year by the Polish Academy of Sciences – Cracow Branch, ul. św. Jana 28, 31-018 Cracow, Poland and the Jagiellonian University in Cracow, ul. Gołębia 24, 31-007 Cracow, Poland

Publisher: Verista: http://versita.com/abcsb
Set and printed by KON Tekst Publishing House, Bobrzeckiej 9, 31-216 Cracow, Poland
Technical editor: Wojciech Marcinek
Author of graphic: Wojciech Pałasz

ACTA BIOLOGICA CRACOVIENSIA Series Botanica on the Internet
The home page of Acta Biologica Cracoviensia Series Botanica can be found at http://www.ib.uj.edu.pl/abc/abc.htm
Webmaster: Piotr Osyczka

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Indexed in Science Citation Index, Current Contents (Agriculture, Biology & Environmental Sciences), Biological Abstracts, BIOSIS Data, Scopus, Index Copernicus, Polish Scientific Journals Contents - AGRIC. & BIOL. SCI., AGRO-AGEN, CABI databases (Agroforestry Abstracts, Botanical Pesticides, CAB Abstracts, Crop Physiology Abstracts, Crop Science Database, Dairy Science Abstracts, Field Crop Abstracts, Forest Products Abstracts, Forestry Abstracts, Grasslands and Forage Abstracts, Horticultural Science Abstracts, Maize Abstracts, Ornamental Horticulture, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Growth Regulator Abstracts, Potato Abstracts, Rice Abstracts, Seed Abstracts, Soils and Fertilizers Abstracts, Soybean Abstracts, Sugar Industry Abstracts, Wheat, Barley and Triticale Abstracts), Foodline Science, AGRIS, AGRICOLA, The Summon

ACTA BIOLOGICA CRACOVIENSIA
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XXX Conference on Embryology
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May 16–18, 2012
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CONTENTS

Volume 54, suppl. 1, 2012

Plenary lectures

Oral presentations

Posters

PLENARY LECTURES

Transcriptional activity of the gametes and zygotic genome activation in flowering plants

Elżbieta Bednarska-Kozakiewicz*, Katarzyna Niedojadło, Krzysztof Zienkiewicz

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In Angiospermae processes and mechanisms involved in gamete differentiation, double fertilization and zygotic genome activation are still poorly explained.

The aim of investigations was to analyze the nuclear activity of sperm cells and female gametophyte cells before and after fertilization in *Hyacinthus orientalis*. Using fluorescence in situ hybridization (FISH) and immunolabelling the spatial and temporal pattern of the total pool of nascent transcripts, poly(A)RNA, polymerase RNAII and rRNA in in vitro growing pollen tubes and in vivo developing embryo sac was determined.

Our study has shown that the sperm cells of *Hyacinthus orientalis* activate their transcriptional activity just after the generative cell division and they undergo gradual inactivation of transcription, both nuclear and nucleolar, at the final steps of pollen tube growth. All these events are interpreted as a complete course of the sperm cell maturation process, and that sperm cells can introduce potentially into the zygote the paternal transcripts.

The investigations of female gametophyte have revealed that mature egg cell of *Hyacinthus orientalis* is almost completely transcriptionally silenced. Only a small pool of RNA Pol II and splicing factors is present in its nucleus and it does not accumulate large amounts of poly(A) RNA. On the contrary nucleolar activity of the female gamete is directed at the accumulation of rRNPs in the cytoplasm and of immature rRNA in the nucleolus. The nuclear activity of the central cell is slightly higher and accumulation of rRNPs in cytoplasm and pre-rRNA in nucleolus take place. The transcriptional silencing of the cells that will soon undergo fertilization remains through the progamic phase. The fertilization initiates the maturation of maternal pre-rRNA and induces the activation of the zygote and primary endosperm cell genomes. Just after fertilization the strong increase of the nascent transcripts, as well as poly(A) RNA and polRNA II was observed in both fertilized cells. The presence of second nucleolus in zygote and third in endosperm cells then reflect the resumption of the expression of zygotic rDNA.

These results we discuss against the topical study on molecular bases of fertilization in Angiospermae.

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Higher Education, grant N N303 290434.

The features of the female reproductive system in leeches (Hirudinida) in an epistemological-evolutionistic concept

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Taxonomy of leeches has been strongly influenced by various research traditions, but it still presents numerous problems to researchers. The aim of the study was to test the hypothesis that the features of female reproductive system are predictive of phylogenetic reconstruction of Hirudinida at the family level. In our study we have used 18 characters associated with the female reproductive system, the ovary organization and the course of oogenesis in 11 representatives of leeches belonging to five families: Glossiphoniidae, Piscicolidae, Erpobdellidae, Hirudinidae and Haemopidae. *Acanthobdella peledina*, *Branchiobdella parasitica* and *B. pentodonta* were included as an outgroup. All characters were polarized according to the criteria suggested by Sereno (2007) and were used in phylogenetic reconstruction performed with PAUP* 4.06b10 (Swofford, 2000). Parsimony analysis has resulted in one most parsimonious cladogram showing polytomies within Hirudiniformes and Piscicolidae. The latter leech family appeared to be most related to the group containing Glossiphoniidae and *Erpobdella octoculata*. Thus, in our analyses both Rhynchobdellida and Arhynchobdellida appear to be non-monophyletic, what do not completely confirm the results obtained by other researchers in molecular studies (e.g. Siddall and Burreson, 1998; Trontelj et al., 1999). Moreover, some characters, i.e. poorly developed cytophore and apical cell in Erpobdellidae, or yolky eggs in Glossiphoniidae appear to be homoplasies.

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Neuroplasticity in the visual system of *Drosophila melanogaster*

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Neuroplasticity is a term describing a range of adaptive changes that occur in the structure and functions of the nervous system. The visual system of animals is often used as a model to study neuroplasticity. This phenomenon has been reported in the developing and adult organism induced by injury, sensory experience and learning. We have found that neuroplasticity occurs also spontaneously in the adult nervous system and show circadian rhythmicity. Using *Drosophila melanogaster* we have observed that interneurons and glial cells in the first optic neuropil (lamina) of the optic lobe change their morphology during the day and night (Pyza and Meinertzhagen, 1999; Weber et al., 2009). Moreover, the number of synaptic contacts and abundance of the presynaptic scaffolding protein, Bruchpilot (BRP) show daily rhythms. All rhythms which have described in the visual system of *D. melanogaster* are maintained in constant darkness, indicating that they are generated by a circadian clock. They are entrained by light to the daily cycle of light and darkness in the environment. In *D. melanogaster* size of two interneurons in the lamina, L1 and L2, and the number of tetrad synapses increase twice during the 24 h period, at the beginning of the day and night. These rhythms are correlated with the circadian rhythm in locomotor activity and their function is to enhance synaptic transmission between the eye photoreceptors and the first order interneurons during high activity of flies. Both peaks in pre- and postsynaptic neurons are regulated differently, however. In the photoreceptors - presynaptic cells, the morning peak of the rhythms is regulated only by light but the evening peak by the circadian clock. In turn, in the L2 postsynaptic interneurons, cyclical remodelling of their morphology is regulated only by the circadian clock. In result morphological changes in synapses and in dendrites of postsynaptic interneurons are correlated to each other and with external changes of light and darkness. The circadian input to the lamina originates from clock neurons located in the brain which show cyclical expression of a clock gene *period* (*per*). The null per^0 mutants are arrhythmic in locomotor activity and their L2 cells do not show circadian plasticity in morphology. The number of tetrad synapses and BRP level are also constant in the per^0 flies.

ACKNOWLEDGEMENTS: This work was supported by the NCN, grant nr N N301 038240.

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Floral and pollen characters a useful tools in *Viola* taxonomy

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Reproductive organs characters are essential in the genus *Viola* taxonomy (Kuta et al., 2011). The aim of the present studies was to analyze in details using scanning electron microscopy (SEM) the microstructural characters of generative organs (style and stigma, stamens with nectaries) to find evident differences between three sections (Viola, Melanium, Dischidium) and subsections within the Viola section (*Viola, Rostratae, Repentes, Plagiostigma*).

Two main sections Viola (violets) and Melanium (pansies) differ in chasmogamous flower morphology and structure which are an evolutionary traits adapted to pollinators. Flowers in both sections are zygomorphic with five petals, the lower with the spur. Five filamentless stamens surrounded very closely the ovary, two stamens have characteristic elongated connective appendages with nectar-secreting tissue which project into the spur. Flowers of the Viola section are blue, violet, white, sterno- or nototribic with style beaked at the apex glabrous or covered by papillae and/or hairs, depending of subsection. Flowers of the evolutionary young Melanium section are multicolored, nototribic, with style ended by cup-shaped stigma covered with papillae and hairs with an hole on the top and a typical lip below the hole. Species of sect. Viola develop during the season chasmogamous, semi-cleistogamous and cleistogamous flowers whereas no cleistogamy in the Melanium section. *V. uliginosa*, the solely representative of the *Repentes* subsection, develops cleistogamous flowers as was confirmed by SEM analysis therefore is not an exception in the Viola section as was emphasized previously in European floras. *Viola biflora* of Dischidium section with yellow petals directed upwards as in section Melanium differs conspicuously from this section in stigma shape and structure. Style has characteristic 2-lobed stigma. Pollen is heteromorphic with more than three apertures in Melanium section, mainly with three apertures in Viola section.

In conclusion: Style and stigma structure are essential characters for distinguishing two main sections - Viola and Melanium and maintain *V. biflora* in its own section Dischidium not including it into Melanium section. *V. uliginosa* as other species of Viola section develops cleistogamous flowers.

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Androgenesis and stability of regenerants

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Induced doubled haploidy (DH) has become the most widely used and efficient method to produce homozygous breeding lines from heterozygous material in a single generation. Of the several methods used for DH production, androgenesis is technically easy and can be applied to a wide range of species. Breeding with the DH system requires regeneration of a sufficiently large number of fertile plants. Regardless of the genotype and technological differences, the obtained DH lines are expected to be genetically and morphologically uniform, not segregating, so that any variability within the lines may only be environmental in nature. However, as experience shows, both the quality of the starting material and the in vitro culture conditions can affect the stability of DH plants. The procedures may disturb the cell cycle, change DNA content, and induce abnormalities in the structure and the number of chromosomes (aneuploidy). Such genetic and chromosome disorders may occur independently of each other and the mechanisms behind them are not entirely clear. Deviation from stability is detrimental and in most cases disqualifies produced material at various stages from further research and breeding. Additional factors that reduce yields of androgenesis are the presence of clones and high proportions of albino plants following regeneration. All phenomena not only reduce the regeneration efficiency that interferes with proper evaluation of generated progenies, but increase costs and workloads of their production. Therefore, successful application of DH technologies requires careful consideration and planning to develop balanced and effective strategies.

Triticale is one of the easiest cereals to induce androgenesis in and to produce adequately sized populations of DH lines. Because DH lines in triticale are usually made from F1 hybrids, the problem of instability or variability among triticale regenerants appears much more important compared to other crops.

Evolution of viviparity – from Placodermi to eutherians

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During over 430 mln years of evolution of vertebrates viviparity has arisen independently in many lineages: e.g. in chondrichthyan and osteichthyan fishes – 22 ×, in amphibians – 6 ×, in reptiles – 108 × and in mammals – 2 ×. In such groups as mammals, chondrichtyans and caecilians viviparity is a dominant reproductive strategy, whereas in reptiles, where has originated in over 100 separated lineages, often at low taxonomic levels and in geologically recent times – exists only in about 20% species of lizards and snakes Blackburn, 2005).

Recently the viviparity has been documented also in many extinct groups: placoderms, plesiosauria and ichtyosauria. The discovery of viviparity in fossil placoderms has implications, which may suggest, that insemination and livebearing has originated early in vertebrate evolution, because this strategy was very popular in devonian period (Long et al., 2009).

The independent evolution of viviparity involved enormous variety of adaptations in maternal-fetal relationship and modifications both maternal (oviduct, uterus, internal ovarian epithelium and follicle) and embryonic tissues (body surface epithelium, buccal, gill and gut epithelium) (Pecio, 2001). Some of the structure e.g. yolk sac placenta, placentas, facilitating the transfer of waste material and nutrients evolved convergently and "*there are as many evolutionary scenarios in the literature as that of the evolution of viviparity*" (Wake, 1992).

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In vitro development pathways of isolated endosperm

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The endosperm is a tissue unique in its origin, development and ploidy level. In most angiosperms it is a product of the fusion of a haploid sperm nucleus with two haploid polar nuclei. This fusion results in a triploid structure, the endosperm, which develops into a tissue consumed by the embryo. There are no reports concerning in vivo endosperm differentiation. However in vitro conditions opened new perspectives to investigation this specific plant tissue. It was proved that isolated endosperm of different species has the potential to proliferate, differentiate, and finally regenerate plants.

Experiments conducted on isolated endosperm are important as well for basic knowledge as for agriculture practice. In our previous works the endosperm-derived callus in kiwifruit was used to investigate the plant extracellular matrix and differences between morphogenic and non-morphogenic callus. As practical use the endosperm culture could be a method for triploid plants obtaining. Successful 3C plant regeneration from endosperm-derived callus has been reported e.g. in acacia, kiwifruit, walnut, papaya.

As well immature as mature endosperm tissues are able to proliferate under in vitro conditions. However successful plant regeneration depends on many factors like proper stage of endosperm development and culture conditions. Also a capacity for proliferation and morphogenic response differ among species. There is an interesting relation between nutrition status of plant and a kind of organogenesis pathway. Parasitic and semi-parasitic species showed direct organogenesis, whereas the endosperm of autotrophic plant indicated indirect organogenesis and callus tissue is necessary for next steps of differentiation.

On the other hand immature endosperm could continue development in similar way like in planta, what we observed recently in some cereals. The regulation of cereal endosperm development is still far from understood. Filling this knowledge gap may provide a means to manipulate the development of this tissue and the caryopsis of important crops. There are no detailed histological studies of the in vitro development of endosperm isolated from cereals. Our investigations conducted on endosperm of bread wheat, durum wheat and triticale open new possibilities in experimental plant embryology.

Cell elongation, endoreduplication and nuclear movement during germination and collet hair formation in *Arabidopsis thaliana*

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Seed germination and early seedling growth of *Arabidopsis thaliana* include elongation of the epidermal cells of the root-hypocotyl transition zone (the collet) and highly synchronous development of hairs. Epidermal hairs formed in the collet region anchor the seedling to the substratum and facilitate geotropic responses and water uptake well before the development of root hairs, but this region has largely been ignored in research on germination and the early establishment of the seedling. Using laser-scanning confocal microscopy of fluorescent-labelled *Arabidopsis* embryos during and following germination, several movies were made which show the cellular changes that occur during emergence of the radicle and the dynamics of collet hair growth and nuclear migration therein. Endoreduplication was estimated by flow cytometry. Germination is completed by expansion of cells of the root-hypocotyl transition zone/the collet that is immediately behind the radicle (Sliwinska et al., 2009). From this region there is the simultaneous initiation of hairs following the completion of germination; their growth is accompanied by the synchronous migration of nuclei at a short fixed distance behind the tip. However, disruption of nuclear movement in the growing collet hairs using low concentrations of cytoskeletal inhibitors or the *root hair defective3* (*rhd3*) mutant, demonstrated that nuclear position within a cell is not a prerequisite for tip-directed growth of the hair, but it is required for correct tip elongation. Following cessation of hair growth, the nuclei migrate to the base of the cell; thereafter their movement is limited and asynchronous. This is due to the inability of most nuclei to migrate past large vesicles (pre-vacuoles) that form in the mature hairs. Changes in nuclear dynamics are accompanied by an increase in nuclear DNA content due to endoreduplication, from 4C to 16C.

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Biogenesis and function of the Cajal body

Dariusz J. Smoliński[1*], Agnieszka Kołowerzo[1], Michał Świdziński[1]

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The spatial organization of the cell nucleus plays a fundamental role in genome function and maintenance. The nucleus is a complex organelle containing numerous dynamic domains. These nuclear suborganelles lack defining membranes and are collectively referred as nuclear bodies (NBs). Many of NBs carry out specific nuclear functions. Many important nuclear processes are organized in discrete subdomains, such as the synthesis and processing of pre-ribosomal RNA, DNA replication and repair, the storage and assembly of spliceosomal components, the histone mRNA maturation. One of the most extensively studied nuclear bodies, the Cajal body (CB), was originally identified more than 100 years ago. CBs are evolutionarily conserved structures, enriched in components of the RNA processing machinery. Although Cajal bodies are found in the majority of animal and plant cell types not all cell types contain CBs. CBs are most prominent in transcriptionally active cells. Thus cells with CBs must have a physiological need for their existence, although the activities that take place within the CB likely can also occur in the nucleoplasm. In embryological models CB number is regulated during development to respond to the demands of gene expression (Strzelecka et al., 2010; Smoliński et al., 2011). We have investigated in a natural model larch meiocytes distribution of mRNA in comparison to changes in the level of total transcription. Detailed analysis showed that the oval, regular concentrations of mRNAs localized to CBs (Smoliński and Kołowerzo, 2012). This new role of CBs in mRNA metabolism during larch microsporocyte development will be discussed.

ACKNOWLEDGMENTS: This work was supported by a grant from the Polish Ministry of Science and Higher Education no. N 303 799640.

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Cardiac progenitor cells – applications in heart therapy

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Progenitor cells are intermediate form of cells during differentiation of embryonic stem cells into the morphologically and functionally cardiomyocytes. Multipotient cardiac progenitor cells were found both in fetal and adult heart of many mammals and humans.

Stem cells derived from early embryos can be induced to undergo via mesoderm to cardiac progenitor cells expressing GATA-4, Nkx2 and Isl-1, finally, they differentiate into cardiomyocytes. Alternative source of embryonic stem cells are skin fibroblasts that could be converted to iPS cells. Both groups of cells could be useful in researches on human heart diseases and allowing the creation of accurate models of genetic diseases and congenital malformations. Progenitor heart cells used in therapy give stable integration with myocardium, decreased risk of cytotoxicity and graft rejection and sustained contractile activity. Additionally, paracrine action of transplanted cells and induced vasculogenesis may improve function of injured myocardium.

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Embryo and endosperm development of hybrids from the crosses of chosen *Brassica* species

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Rapeseed (*Brassica napus* L.) is an amphidiploid (2n=AACC) evolved from two diploid ancestors: *B. campestrsis* (2n=AA=20) and *B. oleracea* (2n=CC=18). In the end of twenty century this species has become a leading oil crop of Europe, Canada, China and Australia. Artificial synthesis of rapeseed through crossing of these two diploid species has been carried for almost eighty years and was undertaken by various authors (U, 1935; Ollson, 1960; Inomata, 1977; Wojciechowski, 1985). Wide hybridization has been and still is a useful method for plant breeders to create new plant forms with introduced valid genes from related species. Plant material used in this study consisted of F_2 and F_3 interspecific hybrids from the crosses of *B. napus* with two diploid (*B. campestris* ssp. *pekinensis* and ssp. *trilocularis*) and two amphidiploid forms (*B. carinata*, 2n=BBCC=34 and *B. juncea*, 2n=AABB=36). In these hybrids the observations of embryo and endosperm development in comparison to rapeseed cultivars were conducted. It was found that the development rate of embryo in hybrid plants was different depending on their origin. In *B. napus* × *B. campestris* hybrids development of embryo was faster than in rapeseed cultivar and than in *B. napus* × *B. carinata* and *B. juncea* hybrids. Generally, siliquae of hybrid plants showed lower number of ovules compare to rapeseed cultivar and higher percentage of ovules without embryo. The reason for that phenomenon was lack of endosperm or its defective development.

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Current challenges for GMO applications in Europe and in Poland

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The goal of agriculture has always been to domesticate plants and animals for the good of man. All the contemporarily grown crops originate from wild plants that were changed by people for thousands of years. Genetic engineering represents an extension of this process. It has the potential to increase yields and allow crops to be grown under conditions that currently limit plant development.

The number of countries growing GM crops in 2011 reached 29. Although it seems that the use of GM crops is expanding rapidly, there is still a very controversial discussion around potential environmental and health risks and unclear benefits.

The establishment and implementation of a biosafety strategy was a task of European and national administrations. A rule called the "precautionary principle" has become the central dogma of decision makers in the European Union.

The precautionary principle is applied in situations that may expose the society or environment to unacceptable risks. The principle gives risk managers a possibility to act responsibly even in the face of scientific uncertainty.

International commitments undertaken by Poland set a need for a law concerning GMOs, which would be in compliance with international standards. Currently a project of a new law on GMOs is being prepared. According to proposed changes, it will still be possible formally to release GMOs into the environment and place GM products on the market. However, the government issued its "framework position" concerning GMOs. According to this position, the government is against the deliberate release of GMOs into the environment, but is not against the introduction of GM products other than food and feed into the market and placing of GM food on the market, unless it is imported from outside or from EU and was not being processed on Polish territory. The proposed changes also introduce new restrictions to the release of GMO in to environment. On the other hand we are dealing at the moment with increasing damage of corn crops by two pests European corn borer and western corn rootworm in Europe and in Poland. These two pests cause heavy losses in yield and elevate the cost of treatment, whereas the area of corn cultivation is constantly growing.

ORAL PRESENTATIONS

The comparison of processes of cell regeneration in the midgut epithelium of *Lithobius forficatus* and *Scolopendra cingulata* (Myriapoda, Chilopoda)

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The regeneration is one of the most important phenomena in organisms, because it enables the maintenance of homeostasis. It allows the reconstruction of damaged or lost cells, tissue and organs, and also the remodelling the body. Therefore it is one of the important processes observed during the embryogenesis, post-embryonic development and in adult organisms.

One of the organ which is treated as the model one in analysis of processes of proliferation and differentiation is the endodermal part of the digestive system (midgut) of invertebrates. In Myriapoda, as in many invertebrates the midgut is able to regenerate due to the presence of regenerative cells (called as "midgut stem cells").

In both examined species (*Lithobius forficatus* and *Scolopendra cingulata*) the pear-shaped regenerative cells are individually distributed along the entire midgut epithelium between digestive and secretory cells. They are characterized by large, irregular nucleus and the cytoplasm, which is poor in organelles. However, the midgut regenerative cells of *S. cingulata* are much higher than the cells of *L. forficatus*, so their nuclei are not located in the basal part of the midgut epithelium but in the perinuclear or apical cytoplasm. In both cases stem cells divided in a asymmetric way. Proliferative activity of these cells is significantly different in both studied species. In *S. cingulata* midgut regenerative cells are able to divide only in the night, since theirs activity is correlated with circadian cycle and it undergoes in a cyclic manner. However, in *L. forficatus* regenerative cells divide in a continuous manner during the entire lifespan.

The ultrastructure of the midgut regenerative cells was described with the use of transmission electron microscope, while the process of cell proliferation was confirmed by BrdU staining and using the anti-phosphohistone H3 antibody.

Embryological study of the new interspecific hybryd between *Fragaria* × *ananassa* Duch. and *Fragaria vesca* L.

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The present study was undertaken to characterize the development of the ovule of the new interspecific hybrid between *Fragaria* × *ananassa* and *Fragaria vesca*. We obtained this strawberry hybrid after cross-fertilization of cultivated strawberry (*F.* × *ananassa*) (maternal parent) and wild strawberry (*F. vesca*) and in vitro supported germination and micropropagation of the most viable seedling raised from hybrid seeds (Dziadczyk at al., 2007). Hybrid plants bear well-developed, flavorous fruits, produce seeds, and exhibit proper vigour. They are therefore an interesting object for basic research and plant breeding.

Light microscopy investigation of semithin sections revealed that the development of hybrid ovules begins with formation of the nucellus and one integument. The characteristic feature during nucellus development is differentiation of a multicellular archespore. In some ovules, we observed formation of two nucelli inside one ovule. We did not observe such a phenomenon in the ovules of cultivated strawberry (*F.* × *ananassa*) (Dziadczyk at al., 2011). The most characteristic feature during embryo sac development in hybrid ovules is the occurrence of various ways of development. The embryo sacs can originate from meiotic cells and also from unreduced somatic cells developing into apomictic embryo sacs.

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Differentiation of follicular cells in polytrophic ovaries of *Osmylus fulvicephalus* (Neuroptera: Osmylidae). An exceptional case of breaking symmetry during neuropteran oogenesis

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Neuropteran ovaries are composed of several elongated polytrophic ovarioles which consist of a few easily recognizable zones: a terminal filament, germarium and vitellarium. The vitellarium contains several egg chambers in a linear arrangement and progressively advanced in oogenesis. Each egg chamber is formed by a cluster of germ cells covered by a single layered somatic follicular epithelium (Kubrakiewicz, 1997; Garbiec and Kubrakiewicz, 2012). During subsequent stages of oogenesis an initially uniform population of follicular cells diversifies into several subpopulations that exhibit different behavior and perform different functions. One of them forms an prominent epithelial fold on the anterior pole of the oocyte (Garbiec and Kubrakiewicz, 2012). During choriogenesis (the last stage of oogenesis) cells of the anterior fold produce aeromicropyle, a specialized region of a chorion (Kubrakiewicz et al., 2005). In most neuropterans the anterior fold is radially symmetric and so is the aeromicropyle. Ecceptionally, in *O. fulvicephalus* follicular cells forming the anterior fold are shifted off the anterior-posterior axis of the egg chamber to a ventral position. In consequence, the aeromicropyle is formed eccentrically. Moreover, the anterior epithelial fold and the aeromicropyle are internally asymmetric. Follicular cells, projections of which mould micropylar canals are located exclusively on the ventral side of the fold. Rearrangement of the follicular cells of the anterior fold in *Osmylus* egg chambers is preceded and accompanied by a change in the position of the oocyte nucleus. Just before the epithelium of the anterior fold becomes asymmetric, the oocyte nucleus migrates from a central to dorsal position within the ooplasm. The occurrence of both events is probably not coincidental. Probable mechanisms underlying their interrelationship are discussed.

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Developmental competence of pig embryos after standard in vitro fertilization or intracytoplasmic sperm injection (ICSI)

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The production of porcine embryos in vitro is still relatively inefficient. The main reasons for this limited performance are polyspermy after in vitro fertilization and the poor developmental ability of obtained zygotes. Intracytoplasmic sperm injection is the possible solution which excluded polyspermy. The aim of this study was to compare the developmental competence of pig zygotes derived from standard in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Cumulus oocytes complexes (COC's) were obtained by aspiration from antral follicles of ovaries collected from slaughtered gilts. Then the oocytes were cultured in modified TC-199 medium to metaphase II for 42 hours. Semen for IVF was incubated in modified capacitation medium – M199 for 1 h. Sperm fraction was introduced to the droplets containing oocytes and next gametes were co-incubated for 4h in modified TC-199 medium. ICSI was performed using mechanical micromanipulator (Research Instruments Limited, Cornwall, England). Micromanipulation was carried out in modified NCSU-37 medium. The tail of spermatozoa was broken and then single spermatozoa was aspirated into the injection pipette. The oocyte was fixed by the holding pipette and next sperm head was introduced into the oocyte cytoplasm. Presumptive zygotes were cultured in vitro for 144 h in NCSU- 23 medium. Embryo quality criteria were cleavage, morula and blastocyst rates. The experiment was performed on 136 zygotes obtained after IVF and 83 zygotes obtained after ICSI. The percentage of embryos developed to the morula and blastocyst stage were 42,3 and 28,8% after IVF, respectively and 51,7 and 34,5% after ICSI, respectively. Our preliminary studies demonstrated higher developmental competence of ICSI derived embryos compared to standard IVF produced ones.

ACKNOWLEDGEMENTS: This study was funded by project N N311 516140 by NCN, Poland.

LM and SEM study of the prenatal development of the palate in domestic cat

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The developmental changes of the mucosa of the hard and soft palate after fusion of the lateral palatine processes in the fetuses of the domestic cats were studied means LM and SEM methods. We used for study the fetuses ranging from 38 to 63 day p.c. and the adult cats.

The aim of study was to describe the development of the mucosa structures as palatal raphe, transverse palatal rugae, and palatal glands. We observed also the process of differentiation and keratinisation of the palatal epithelia.

On 36 day p.c. the palatal raphe is clearly visible on surface of hard and soft palate. On the hard palate there are 6 primordial transverse rugae curved towards pharynx. The raphe start to disappear from incisive papillae area and at 50^{th} day p.c. is observed only near last palatal rugae and on soft palate.

On 45 day p.c. on first palatal rugae appear the primordial of conical papillae. The papillae develops gradually from palatal raphe to the lateral sides of rugae. On the end of prenatal period the papillae form anterior and posterior rows on each palatal rugae. The most number of papillae is on 5^{th} rugae, which correspond the structure of the tongue.

In the development of palatal mucosa we distinguished three stages. From 38 to 45 day p.c. the undifferentiated epithelium cover the mesenchymal tissue. Around 50 day p.c. the epithelium start to differentiate into multilayered and the mesenchyme change in loose connective tissue. On this time palatal rugae change the orientation towards pharynx. From 55–63 day p.c. the epithelium starts to keratinize. The development of the glands in soft palate start from 45 day p.c. These primordia develop into fully active secretory units at 55 day of p.c.

Morphogenesis and translocation of nuage complexes in the *Drosophila* ovarian follicles

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Assembly and subsequent localization of ribonucleoprotein (RNP) complexes in distinct regions of the oocyte cytoplasm are universal features of animal oogenesis and early embryonic development (Kloc and Etkin, 2005; Holt and Bullock, 2009). Ultrastructural studies revealed that the cytoplasm of the *Drosophila* germ-line cells, i.e. oocytes and nurse cells, comprises characteristic accumulations of electron-dense, granulo-fibrilar material termed nuage, which contain RNPs and participate in the formation of the polar/germ plasm (reviewed in Mahowald, 2001). The precise mechanisms responsible for transfer of nuage accumulations from nurse cells into the oocyte, and their subsequent migration within the ooplasm, is still not fully understood. Using modified standard and immunogold electron microscopy methods, we followed the origin and developmental stages of nuage accumulations within the nurse cell-oocyte syncytium in the ovary of the fruit fly, *Drosophila melanogaster*, at the ultrastructural level. We demonstrated that two morphologically and biochemically distinct forms of the nuage material exist in the nurse cell cytoplasm: 1) translocating complexes of nuage containing Vasa protein, termed sponge bodies, and 2) stationary, polymorphic accumulations of nuage enriched in Argonaute and Survival of motor neuron proteins. Immunogold labeling combined with the confocal fluorescent and ultrastructural analyses revealed that the Vasa-containing nuage complexes remain closely associated with the cisternae of the endoplasmic reticulum throughout their lifetimes and migrate by a mechanism, which is quite distinct from the microtubule-dependent translocation of other nuage-like complexes i.e. *oskar* RNPs. We suggest that two separate nuage translocation pathways participate in the formation of the polar granules within the germ plasm of the oocyte posterior pole. We also provide morphological and immunocytochemical evidence that the polymorphic nuage accumulations occurring in the nurse cell cytoplasm correspond to the recently identified domains termed U body-P body complexes.

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Differentiation of somatic cells in the ovary of pseudoscorpion *Chelifer cancroides* (Linneus, 1761) and their contribution to oogenesis and early embryonic development

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We followed changes in the spatial organization of the somatic cells during subsequent stages of the ovary development in a pseudoscorpion, *Chelifer cancroides*. In young females the ovary is a solid cylinder. Its interior is filled with germline cells (oogonia and early meiotic oocytes) and undifferentiated inner somatic cells. The ovarian wall consists of somatic epithelial cells that form a simple epithelium externally covered by the basement lamina. With the progress of previtellogenic growth the oocytes bulge to the hemocoel cavity accompanied by the interstitial cells, which transform into follicular cells surrounding growing oocytes. Oocytes protruding to the hemocoel cavity elevate the epithelium of the ovarian wall which thus form the oocyte external cover. Concurrently, the ovary transforms from a solid cylinder into a tubular structure with a lumen inside. The follicular cells are characteristically arranged over the oocytes. They are flat and elongated. Their long axes align along the proximal-distal axis of the oocyte and so they look like closed flower petals enclosing the oocyte. The most distal tips of particular follicular cells meet at the most distal pole of the oocyte, while their cell bodies sit at the oocyte proximal pole. The cells of the ovarian wall, supported externally by the basement lamina, initially cover the whole surface of the protruding oocytes together with their follicular envelope. With the oocyte growth the ovarian wall cells become progressively and significantly stretched. Initially they form extended and thin projections that embrace the oocytes. Eventually the ovarian epithelial cells slip off the oocyte and can be found only at its base (proximal pole) forming an "eggcup" supporting structure, the stalk. The stalk is a tubular structure. Its wall consists of a simple epithelium, while its lumen opens into the lumen of the ovary. At advanced vitellogenesis the stalk cells enlarge and form a simple columnar epithelium. Stalk cells secrete granular material deposited onto the oocyte surface during ovulation. After ovulation the stalk cells undergo hypertrophic growth and secrete a nutritional fluid provided for developing embryos. The follicular cells gradually degenerate. Their role during oogenesis remains unclear.

Comparison of the embryonic development of spined loach *C. taenia* Linnaeus, 1758 and progeny of allotriploid *Cobitis* females (Pisces, Cobitidae)

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The spined loach *C. taenia* appears in diploid and diploid-polyploid populations dominated by allotriploid *Cobitis* females. Their dominance is not fully explained (Juchno et al., 2007) and apart others may be connected with the period of embryonic development. We present the comparative studies of the embryonic development of *C. taenia* from diploid and of polyploid progeny of triploid *Cobitis* females from mixed populations. Diploid embryos were obtained in the crosses between females and males of *C. taenia* whereas polyploid embryos in crossing between triploid *Cobitis* and the males of some coexisting taxa, *viz. C. taenia*, tetraploid *Cobitis* and golden loach *S. baltica* Witkowski, 1994. The ploidy level of the parents was identified karyologically.

First cleavage started in 1 hour and 40 min. (diploids) and in 1 hour and 30 min. (polyploidy), then the next cleavages appeared after 20 – 30 minutes. Gastrulating took place between 11 and 15 hours, and about 12 hours, respectively in diploid and polyploid embryos. In total the period of diploids development was shorter than of triploids and hatching was observed 50 and 58.5 hours after fertilization, respectively. All newly hatched larvae were pigmented and (in one day) had external gills. On the third day after hatching the larvae of *C. taenia* were significantly smaller than those of triploids *Cobitis*.

Percentage of larvae and their mortality were similar. In average, hatching was observed in 48% and 42% of all eggs of respectively *C. taenia* and of triploid *Cobitis* females. Respectively, 70% and 79% of them attained the age of 17-days.

Lack of fertilizing ability was observed of tetraploid males; the eggs of triploid *Cobitis* activated with their sperm have not been developed. The progeny of 3n *Cobitis* × *S. baltica* lived only 3 days after hatching and characterized by body abnormalities.

ACKNOWLEDGEMENTS: This work was partially supported by the Ministry of Science and Higher Education (grant N N303 068834).

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Structure and development of the female gonad in psyllids (Insecta, Hemiptera: Psylloidea)

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The structure and development of the female gonad in six species of psyllids belonging to the family Psyllidae, *Psylla mali, Psylla alni, Cacopsylla ulmi, Rinocola aceris, Spanioneura buxi, Cacopsylla crataegi* have been examined.

The gonads of the first larval instar consist of a large number of germ cells that undergo a series of several incomplete divisions resulting in the formation of clusters. Each cluster consists of several dozens of cells termed cystocytes. The cystocytes are arranged into a rosette. In the centre of the rosette a polyfusome occurs. Next, cystocytes differentiate into trophocytes and oocytes. In the last larval instar each cluster elongates and transforms into an ovariole. Gonad of the adult female is composed of numerous short telotrophic ovarioles. The individual ovariole consists of a terminal filament, tropharium (trophic chamber), vitellarium and ovariolar stalk (pedicel) that joins the ovarioles with lateral oviduct. The tropharium is located in the anterior part of the ovariole and contains numerous trophocytes (nurse cells) and several early previtellogenic oocytes (arrested oocytes). Tropharia are surrounded by a single layer of flattened somatic cells of an inner epithelial sheath. Since cells membranes between trophocytes disappear, tropharium becomes syncytial. The trophocyte nuclei are embedded in a common cytoplasm. Arrested oocytes are located in a basal part of trophic chamber. In the vitellarium only single oocyte develops. During vitellogenesis, in the oocyte cytoplasm yolk granules and lipid droplets are accumulated. Each oocyte is surrounded by a single layer of follicular cells that are responsible for the synthesis of precursors of egg envelopes. Egg capsule consists of an internal vitelline envelope and external chorion. The chorion has a complex structure: (1) it is perforated by aeropylar channels and (2) is equipped with long stalk. The latter attaches the eggs to the plant leafs.

ACKNOWLEDGEMENTS: This work was supported by research grant DS/MND/WBiNoZ/IZ/15//2011.

Floral ultrastructural features of *Anacamptis pyramidalis* f. *fumeauxiana*

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Floral spurs are believed as characters influencing on pollinator activities suggesting the existence of a nectar reward. The type form *Anacamptis pyramidalis* is regarded as a deceitful orchid with a nectarless spur. In form *fumeauxiana*, additional one or two spurs were noticed on the lateral sepals (the mutation called neoheterotopy). The secretory function was detected on the adaxial surface of the lip, the lip calli, the tips of the lateral sepals, both epidermises of lip spur and lateral sepal spur. The typical osmophore features as dense cytoplasm with numerous profiles of ER, mitochondria, plastids with plastoglobuli and tubular structures, a large nucleus, lipid droplets, vesicles connected with plasmalemma were examined. The resemblance of the floral morphology between *Gymnadenia conopsea* and *A. pyramidalis* is noticeable: diurnal anthesis, horizontal landing place, pleasant scent, plain nectar guides, moreover the same pollinators (butterflies) and the flowering period. Do we have the food-deceptive pollination mechanism: the imitation of nectar presence *G. conopsea* (as a rewarding model) and *A. pyramidalis* (as a non-rewarding mimics)?

ACKNOWLEDGEMENTS: This work was supported by the University of Gdansk (DS./L150-4-0128-1) and by the National Science Centre in Poland (N N303 580439).

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Regeneration efficiency of doubled haploids related to order of the stem within winter triticale (*Triticosecale* Wittmack)

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Homozygous doubled haploid (DH) lines obtained via androgenesis by in vitro anthers culture are now frequently used in cereal breeding. These include also triticale. The efficiency of DH production is influenced by many factors. The presented experimental data concern regeneration efficiency of six winter triticale in relation to the stem order.

Stems with spikes in the booting stage (DC 43–45, acc. to Zadoks et al., 1974) were collected prior and after exposing to low temperatures. Developmental stages of microspores from four florets of central spikelets were analysed. The regeneration efficiency of triticale genotypes has been described as the number of regenerated plants from the spike of main stem as well as from each tiller spike according to its position.

Regeneration efficiency varied significantly in studied genotypes, from 8.5 to 47.3 regenerated plants/spike. Relation between stem order and regeneration efficiency in each genotype was determined.

Twofold increase in regeneration efficiency from the main spike compared to tiller spikes was reported for the genotype no. 2. Yet for two other genotypes the number of regenerants obtained from tiller spikes i.e. 62.0 (genotype no. 9) and 30.1 (genotype no. 11), exceeds the number of plants derived from the main spike (20.0 0 – genotype 9 and 16.5 – genotype 11). The lowest regeneration efficiency was noted for the genotype no. 1. In two remaining genotypes, the number of regenerated plants from the main and tiller spikes was comparable, i.e. 11 – 12 (genotype no. 5) and 27 – 29 (genotype no. 8) regenerants/spike.

Our aim was to test the efficiency of regeneration, depending on the spike, from which the anthers were used in in vitro cultures. Presented results indicate the variation of efficiency, depending on the genotype.

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The ovary organization and early oogenesis in Remipedia (Crustacea) support the Pancrustacea hypothesis

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Remipedia are enigmatic crustaceans of uncertain phylogenetic position with the general consensus that they are crucial for understanding of the crustacean/arthropod evolution. Remipede bodies house an astonishing combination of both primitive and advanced characters. It seems therefore not surprising that the phylogenetic position of Remipedia within the pancrustacean clade has been a matter of controversy for decades. It has been demonstrated previously that the features of the ovary organization and subcellular aspects of oogenesis are useful in resolving phylogenetic relationships in various arthropods groups. The structure of the female gonads in Remipedia remains largely unknown therefore we have examined gross morphology and ultrastructural details of the ovary in a remipede, *Godzilliognomus frondosus* indicating characters relevant to phylogenetic reconstructions. The ovaries of *G. frondosus* are located in the anterior part of the body and are composed of a single anterior proliferative zone (the germarium) and paired ovarian tubes (the vitellarium). The oocytes undergo subsequent stages of development within the lumen of the ovarian tubes hence the remipede ovaries can be classified as endogenous. During oogenesis, each oocyte is enveloped by a set of characteristic somatic follicular cells, which results in the formation of distinct ovarian follicles. We demonstrate that Remipedia share significant similarities in the ovary organization with Cephalocarida including the anterior ovary location, anteriormost position of germarium and endogenous type of oocyte development. Our results also indicate basic structural similarities between the ovaries of Xenocarida (Cephalocarida+Remipedia) and basal hexapods (Entognatha), which substantiate a sister group relationship between these clades and add extra support for the Pancrustacea hypothesis.

ACKNOWLEDGEMENTS: This work was supported by grant NZ4/00595 from the National Science Centre (NCN).

An effect of different oxygen conditions on the development of the olfactory organ in reference to development of *Amia calva*

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Amia calva is the only living representative of Amiiformes order. It is a species having a swim bladder converted into a lung, allowing it to breathe atmospheric air. It gives a high tolerance to variable oxygen conditions and the ability to survive even in the water almost devoid of oxygen. Aim of this study was to examine whether different oxygen conditions (hypoxia, normoxia and hyperoxia) have an impact on the development of the olfactory organ in A. calva in different age classes. In the study the olfactory rosettes of left-sided olfactory organ of two age classes grown in defined under aerobic conditions were used. After sectioning of rosettes, and then the individual olfactory lamellae, the surface was measured, additionally in specimens from older age class the number of proliferating and apoptotic cells using the method of immunocytochemistry (PCNA and CPP-32) was counted. Both number of proliferating and apoptotic cells showed statistically significant differences between individuals of different oxygen conditions. Individuals reared in hyperoxic conditions showed the greatest rate of proliferation and apoptosis, and the smallest surface of the olfactory lamellae. It demonstrates the significant influence of oxygen conditions on the development of the olfactory organ in this species.

Studies on germline cysts organization in clitellate annelids

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In animals during early gametogenesis, germ cells tend to form syncytial cysts of interconnected cells. Such cysts are formed as a result of incomplete cytokinesis of gonial cells. Each cell in a cyst (i.e. cystocyte) is connected to others cells via one or more stable intercellular bridges (ring canals). The formation of germline cysts seems to be a conserved phase of animal gametogenesis and it has been described in numerous invertebrate and vertebrate species (Pepling et al., 1999). The details of germline cysts development, organization and functioning come from studies on model organisms as *Drosophila melanogaster*, however, such processes are hardly known in other non-model systems. In worms, nematodes, some platyhelminthes and annelids the germline cysts have different architecture than in *Drosophila*. The main attribute of such cysts is the central, anuclear cytoplamsic core to which each cystocyte is connected via only one ring canal (Świątek et al,. 2009) The aim of our studies is not only to find out how such germline clusters are organized but also to explain the role of cytoskeleton in their architecture maintaining. For our studies we chose male germline cysts from an earthworm *Dendrobaena veneta* (Annelida, Clitellata). This species is easy to obtain and to breed in the laboratory conditions, moreover each specimens posses 2 pairs of seminal vesicles, which are filled with germline clusters. To study the effect of cytoskeleton failure on the cysts organization we have developed the method of cultivating germline cysts in vitro. We used special media with addition of cytotoxic substances disrupting the proper functioning of the cytoskeleton. We tested cytochalasin B and D (they affect microfilaments) and colchicine and nocodazole (they affect microtubules). Our studies showed that cytotoxins disrupting cytoskeleton did not change the general pattern of germline cyst organization. However, we observed that the nuclei of germ cells tend to fall into the cytophore via ring canals. Especially, such phenomenon was observed in late spermatogenesis, when the nuclei of late spermatids were usually found within the cytophore.

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Pattern of follicular cells differentiation in polytrophic ovaries in caddisflies (Insecta: Trichoptera)

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The pattern of the follicular cells (FCs) diversification in the ovaries of caddisflies (Trichoptera) is slightly known. In this work we present the results of histological and cytochemical analyses of FCs morphogenesis in three trichopteran species: *Phryganea grandis* (Phryganeidae), *Glyphotaelius pellucidus* (Limnephilidae) and *Hydropsyche* sp. (Hydropsychidae). Paired, meroistic-polytrophic ovaries of caddisflies consist of four to eight ovarioles each. An ovariole can be divided into three parts: terminal filament, germarium and vitellarium. Vitellarium contains a linear array of egg chambers progressively advanced in oogenesis. Linearly arranged egg chambers within the vitellarium are separated by somatic cells forming interfollicular stalks. The cells of the stalk do not divide. Initially they overlap with each other. With the progress of egg chamber growth they flatten and rearrange to form a characteristic stack. Each egg chamber in trichopteran ovaries is composed of eight germ cells (seven nurse cells plus an oocyte) surrounded by a single layer of follicular epithelium. In early previtellogenic egg chamber FCs are diversified into at least two morphologically distinguished subpopulations. Main body follicular cells cover the lateral surface of the oocyte, while some other FCs stretch over the nurse cells compartment. In more advanced stages of oogenesis FCs located at the oocyte/nurse cells border migrate centripetally and eventually enclose the anterior pole of the oocyte. Concurrently, the main body FCs change their shape from cuboidal to squamous. Till the final stages of vitellogenesis main body FCs remain tightly packed and form a coherent epithelial layer.

Endosymbiotic microorganisms of the primitive aphid Sacchiphantes viridis (Insecta, Hemiptera, Adelgoidea: Adelgidae)

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In the body cavity of aphid, *Sacchiphantes viridis*, like in other plant sup-sucking hemipterans, endosymbiotic microorganisms are present. The occurrence of endosymbiotic microorganisms in the insect body is connected with a restricted diet. Phloem sap is rich of carbohydrates but deficient in essential amino acids. It is generally accepted that endosymbionts are responsible for amino acids synthesis and delivery to the host insects. Since endosymbiotic microorganisms are necessary for survival and reproduction of the host insects they are transmitted from one generation to the next transovarially (i.e. the endosymbiotic microorganisms invade female germ cells).

Endosymbiontic bacteria of *Sacchiphantes viridis* are harbored in large, specialized cells termed bacteriocytes. The bacteriocytes are usually integrated into large organs termed bacteriomes. The latter are localized in the close neighborhood of the ovaries. The cytoplasm of bacteriocytes is tightly packed with endosymbiotic bacteria.

Endosymbionts of *Sacchiphantes viridis* are rod-shaped and are surrounded by three membranes, i.e. two own membranes and single, host derived membrane termed the perisymbiotic membrane. The bacteria measure about 2 μm in length and about 1,5 μm in diameter. The bacteria reproduce by binary fission.

In the adult females, the endosymbiotic microorganisms leave the bacteriocytes and migrate to the terminal oocyte. They may migrate both between neighbouring follicular cells or through the cytoplasm of follicular cells. Next, endosymbionts gather in the cytoplasm at the posterior pole of the oocyte.

Identification of violets colonizing ore-bearing areas – from morphology to genes

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Environmental conditions, especially pollution of soils by heavy metals, can affect the plant community composition. In the present investigation, the morphological variability of forest violets (*Viola reichenbachiana* and *V. riviniana*) combined with sequencing of selected molecular markers were studied using samples from four ore-bearing areas (metalliferous populations) in the industrial Cracow-Silesia region with soils rich in heavy metals and from eight non-polluted sites. Both species are morphologically variable when growing under different soil conditions. Intraspecific variability is also increased by interspecific hybrids which are formed when their area of distribution overlap. From field observations, violets growing at polluted areas were morphologically intermediate between *V. reichenbachiana* and *V. riviniana* but were fully fertile. Based on 29 quantitative and 21 qualitative traits, two groups of individuals were separated by principal component analysis (PCA), corresponding to two species – *V. reichenbachiana* and *V. riviniana*. Specimens from two ore-bearing populations have fallen in between, two were included within the *V. riviniana* range.

Chloroplast genome markers *rpoB*, *rpoC1* and *trnH-psbA* were used to discriminate *V. reichenbachiana* from *V. riviniana* and metalliferous populations. Analysis of chloroplast markers *rpoB*, *rpoC1* showed no differences between two species. Non-coding sequence *trnh-psbA*, however was more variable between species. *V. reichenbachiana* populations had high intraspecific variability, manifested as several genetic lineages. *V. riviniana* and metalliferous populations exhibited almost no variability of this particular marker, which suggested that those metallicous forms in respect of this sequence are more similar to *V. riviniana*, than to *V. reichenbachiana*.

In conclusion: violets growing in ore-bearing areas were not affected in their reproduction and can be considered as stabilized introgressive forms toward *V. riviniana* or ecotypes of *V. riviniana* modified by soil conditions.

ACKNOWLEDGEMENTS: This work was funded by the Polish Ministry of Science and Higher Education (project no. 3935/B/P01/2009/36).

The immunocytochemical studies of the egg apparatus in selected species of *Taraxacum*

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Synergids of amfimictic plants are metabolically active cells, involved in the pollen tube attraction. The cell wall at the micropylar contact site of two synergid cells thickens to form the filiform apparatus. Microtubules aggregation near the filiform apparatus was described in some sexual species. They form characteristic brush-like structures oriented in the micropylar-chalazal axis. Our previous observations point to different configuration of the cytoskeleton in synergids in obligatory apomicts in which the embryo and the endosperm developed autonomously without fertilization (Kościńska-Pająk and Bednara, 2006; Kościńska-Pająk et al., 2010).

The aim of the present study was to investigate the configuration of the microtubular cytoskeleton in mature embryo sacs in *Taraxacum udum* (2n=3x=24), the other obligatory apomictic species. To compare the obtained results, similar investigations of sexual *T. linearisquameum* (2n=2x=16) were performed. The visualization of the microtubules was conducted by indirect immunofluorescent microscopy using a monoclonal tubulin antibody. Observations showed that the cytoskeleton of the egg cell in the obligatory apomict *T. udum* is formed by a small number of short microtubules. Spot fluorescence visible in the periphery of the egg cell points to a circular distribution of the microtubules. The cytoplasmic cytoskeleton of synergids was poor, and mostly observed in the micropylar region. However, in the micropylar part of the synergids of the amfimictic *T. linearisquameum*, a higher concentration of microtubules was visible. Obtained results suggest the correlation between the pattern of filiform apparatus and the mode of reproduction.

ACKNOWLEDGMENTS: This work was financed by grant N-N303-807940 of the National Science Centre (NCN).

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Genetic analysis of anther derived diploids of *Capsicum annuum* L.

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The induced androgenesis is the effective biotechnology tool using for rapid genetic stabilization of the hybrid recombinants. Usefulness of the method depends on the androgenic property of species, cultivars or breeding lines. Genotype plays the main role among factors influencing on the practical results of in vitro anther culture. Some of accessions, sweet and big fruited forms are very effective in anther culture. On the other hand, pungent genotypes are, in general, very low responsive. Four F_1 hybrids of *Capsicum annuum* L., the result of crossing soft-flesh, hot, red-fruited lines with hard-flesh, sweet, red- or yellow-fruited cultivars were the donor plants for the induction of androgenesis. Two breeding lines selected from *Capsicum frutescens* L. × *C. annuum* L. hybridization marked "4" and "9" were the maternal forms of donor plants. Polish cultivars 'Luba', 'Mino' and 'Sono' served as a pollen grains source. The initial forms differed in regard to mature fruit colour, pericarp structure and taste. Mentioned features are monogenic. In the general characteristics of hybrid parents three important quantitative characters are also presented. Anther cultures were conduced following the method described by Dumas de Vaulx et al. (1981) for *C. annuum* L. Flower buds were collected from plants grown in plastic greenhouse. Those selected had similar length of sepals and petals. Flow cytometry analysis of plantlets derived in anther culture showed that one third of them had 2C DNA. The diploid plants derived from in vitro anther culture of *Capsicum* spp. F_1 hybrids were the subject of experiment. Thirty five fully developed diploids were under genetic analysis. The mature fruit colour, pericarp structure and taste, conditioned by the marker genes, were the elements of evaluation. Seventy four percent of plants among all examined differed from donor plant phenotype. The differences concerned one (16 plants), two (9 plants) or three (1 plant) features under consideration. The observation gave the unquestionable proof for androgenic origin of these diploids. Spontaneous chromosome doubling occurred probably in the early stage of androgenesis because the plantlet cytometry measurement showed that presence of 1C cells was rare. The remaining diploids, identical to donor plants with regard to marker features, were not similar when compared the fruit morphology.

Retinoic acid and meiosis induction during gonadal development in Anura

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The timing of meiosis induction in fetal gonads, testes or ovaries, depends on a balance between retinoic acid (RA) production and degradation. This sex-related equilibrium is reached in different ways in various Vertebrates. We examined the role of RA in the meiosis induction in six amphibian species by culturing their gonads in the presence/absence of RA and its modulators. We studied the expression of Raldh2 (RA synthesis enzyme) and Cyp26b1 (RA degradation enzyme). In all species Raldh2 was highly active in the epithelium covering the undifferentiated gonads. In the female gonads Raldh2 was detected in somatic cells in the cortex (granulosa precursors), but never found in gonadal medulla. During the testicular differentiation Raldh2 activity decreased or was absent with the exception of *Bombina bombina* testes in which RA was synthesized in spermatogonia. Cyp26b1 protein was found in differentiating Sertoli cells in testes of all the species and only in spermatogonia in *Rana temporaria*. In *Bufo viridis*, Cyp26b1 was expressed exclusively in undifferentiated gonads in parallel with the onset of the Bidder's organ development. Later, the expression of Cyp26b1 ceased in this organ, while was still detectable in the proper gonad. Thus, RA synthesis and degradation underlies the development of the Bidder's organ containing oocytes in both male and female bufonids. Exogenous RA triggered meiosis in larval testes, but not in undifferentiated gonads. RA synthesis inhibition, by using citral, prevented meiosis in differentiating ovaries. Meiosis was also inhibited due to exposure to RAR antagonist blocking the RA receptors. Anti-Raldh2 antibody added to the culture reduced the number of meiocytes in developing ovaries, whereas anti-Cyp26b1 triggered meiosis in the developing testes in all six species. Our results demonstrate that during anuran gonad development: I. the gonad epithelium, somatic cells in the ovarian cortex and kidney tubules synthesize RA, II. RA induces meiosis, III. meiosis in larval testes is inhibited by Cyp26b1 action in the Sertoli cells enclosing germ cells. In conclusion, in anuran gonads the meiosis induction follows a pattern similar to that described in birds and mammals.

Seedling structure and morphology in an "aquatic epiphyte" *Utricularia nelumbifolia* Gardner

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Utricularia species are example of the botanical "misfits", because they do not have a conventional morphology of the vegetative organs. Moreover, most species of this genus have simplified embryos that form a mass of barely differentiated cells without lateral organs. Only a few *Utricularia*, which grow in bromeliad tanks, possess mature embryos with multiple photosynthetic lateral organs that are octopus-like in shape (e.g. Płachno and Świątek, 2010). One of these species is Brazilian *Utricularia nelumbifolia*. Here, we focused on the germination of the *Utricularia nelumbifolia* seeds and the morphology of its seedlings. We also check our hypothesis; that glands on the primary embryo organ absorb solutes from the external environment and fulfill a role similar to that of root hairs, which are absent in *Utricularia*.

ACKNOWLEDGEMENTS: This study was funded by grant N N304 002536 from the Polish Ministry of Science and Higher Education (NCN). B.J. Płachno gratefully acknowledges the scholarship for Outstanding Young Scientists from the Minister of Science and Higher Education.

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The oogenesis of *Macrobiotus polonicus* (Tardigrada, Eutardigrada, Macrobiotidae)

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The female reproductive system of *Macrobiotus polonicus* (Tardigrada, Eutardigrada, Macrobiotidae), as in others Eutardigrada (Poprawa, 2005a) consists of the single ovary and the oviduct that opens into the rectum. The sac-like ovary is located in the dorsal part of the body. Its anterior part is suspended to the dorsal body wall by two ligaments. The ovary is filled with the developing oocytes, which are assisted by the trophocytes. In *Macrobiotus* polonicus, as in other Eutardigrada species (Poprawa, 2005a), mixed vitellogenesis takes place. One part of the yolk material is produced inside the oocyte (autosynthesis), the second part is synthesized in the trophocytes and is transported to the oocytes through the cytoplasmatic bridges. At the end of vitellogenesis oocytes are filled with heterogenous spheres of yolk material.

The egg capsule of tardigrades is composed of two shells: the vitelline envelope and the multilayered chorion (Poprawa, 2005b, 2011). The process of egg capsule formation of *Macrobiotus polonicus* begins at the midpoint of vitellogenesis. The chorion is formed first, then the oocyte secretes material that forms the vitelline envelope. The vitelline envelope is thin and electron dense. The chorion consists of three layers: (1) the inner, homogenous of medium electron density; (2) the thin middle labyrinthine layer; (3) the outer, homogenous, of medium electron density. The chorion surface is covered with conical processes. Their tips are flattened and arranged into rosette. The surface of the conical processes and the basic chorion is smooth.

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Prenatal development of the uterus in domestic cat: preliminary LM and SEM study

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During mammals embryogenesis, the female reproductive tract is formed as a part of the urogenital system, which is derived from the intermediate mesoderm. At the time of early embryonic development of the urogenital system, the embryo contains two pairs of ducts- Wolffian ducts, which are first formed from the intermediate mesoderm, and Müllerian ducts, which arise as invaginations of coelomic epithelium. Further development of both of these ducts depends on the sex of the embryo. In female embryos Wolffian ducts disappear, while Müllerian ducts differentiate into the oviduct, uterus, cervix and vagina. The prenatal development of the uterus begins with formation and than fusion of the Müllerian ducts.

The aim of the study was to investigate the prenatal development of the uterus in domestic cat, using techniques of LM and SEM microscopy. The study was carried out on cat's fetuses aged 29–63 day p.c. and also on juveniles and adults cats.

Prenatal development in cat lasts 63–70 days. On the 21st–23rd day pc Müllerian and Wolffian ducts begin to grow. The studies revealed that on the 29th day pc Wolffian ducts are well-developed and created by simple cuboidal epithelium. In the primordium of the uterus Wolffian ducts run close together, surrounded by undifferentiated embryonic tissue. The Müllerian ducts are smaller than the Wolffian ducts, and created by high epithelial cells, which are arranged in one or two layers. On the 38th day pc the both uterine horns, consist of mesenchyme, which surrounds single Wolffian duct an Müllerian duct. The uterine body has a pair of Wolffian ducts, and a pair of Müllerian ducts. Between the 38th day pc and the 41st day pc, in the uterine body, the Müllerian ducts merge into one duct. The Wolffian ducts gradually fade and on the 50th day pc, they are not observed in the uterine horns and uterine body. About the 50th day pc, in the uterine epithelium, appear vertical intussusceptions, and after that the mesenchyme tissue begin to differentiate into mucosa, muscle and serous layer. Mesenchymal cells in the mucosa have loose arrangement and cells in the muscle layer have spindle-like shape and are arranged circularly. The studies revealed that in cat, the final maturation of the uterus, that include the uterine glands formation, take place after birth.

In vitro conditions induce changes in DNA methylation and autonomous endosperm development in wild genotype and mutants of *Arabidopsis thaliana*

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Double fertilization is usually required to full seed development. These events are circumvented in apomictic plants that reproduce clonally through seeds, sporadically in sexual Angiosperms and under experimental conditions. The molecular research of mechanisms controlling autonomous endosperm development in *fie* and *met1* mutants of *Arabidopsis* strongly suggest, that changes in genomic imprinting and DNA methylation are essential for autonomous/apomictic development (Curtis and Grossniklaus, 2008; Köhler and Weinhofer-Molisch, 2009).

Arabidopsis thaliana genotypes (wild, *met1* and *fie* mutants) were chosen to investigate the development of autonomous endosperm (AE) in unfertilized ovules cultured in vitro and to understand the mechanism controlling AE induction. Unpollinated pistils were cultured on hormone-free Murashige and Skoog medium (MS) with addition of 6% sucrose and supplemented with combinations of phytohormones, mammalian sex hormones or demethylating substance.

AE was induced and developed in wild and mutant genotypes on all media used with the frequency in ovaries reaching 35%. AE induction was strongly depended on genotype but not on type of medium. High frequency of AE induction and advanced development in homozygous mutant (*met1*/*met1*) were probably caused by accumulation of global demethylation (hypomethylation as an effect of *met1* mutation and in vitro condition). The most advanced AE development was observed in *FIE*/*fie* mutants.

In conclusion: The changes in DNA methylation (in one or few genes), caused by in vitro conditions, resulting in AE induction and/or further AE development, suggest that *Arabidopsis thaliana*, a model plant, has an ability to autonomous endosperm development which could be treated as the first step toward apomixis.

ACKNOWLEDGEMENTS: This work was supported by project No N30300731/0281, financed by Polish Ministry of Science and Higher Education.

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The role of cell death in the epithelium of digestive system in juvenile and adult specimens of *Piscicola geometra* (Annelida, Hirudinea)

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The digestive system of both, the juvenile and the adult specimens of *Piscicola geometra* is composed of foregut, midgut and hindgut. The endodermal portion (midgut) possesses four distinct regions: the esophagus, the crop, the posterior crop caecum and the intestine. Their epithelia are formed by flat, cuboidal or columnar digestive cells; however, single small cells which do not contact the midgut lumen were also observed (Rost-Roszkowska et al., 2011).

Digestive cells undergo continuous degeneration: necrosis, apoptosis and autophagy. While necrosis was observed in esophagus, the apoptosis and autophagy together with necrosis occurred in the crop, the posterior crop caecum and the intestine.

In the majority of digestive cells of adult specimens, the formation of autophagosomes and autolysosomes appeared as the common process what might be connected with the cell survival. Midgut cells being in contact with toxic haem must have organized some mechanisms which enable the cell to survive. It is confirmed by the fact, that autophagy was not observed in juvenile specimens of *P. geometra* which did not eat and were not exposed at toxic haem. In several specimens of adult specimens the process of apoptosis occurred, but it mainly concerned digestive cells of the posterior crop caecum and the intestine. In some of the digestive cells which die in an apoptotic way, numerous autophagosomes were observed, while autolysosomes and the residual bodies did not occur. It suggested that initially the autophagy tries to protect the cell against the cell death, but eventually the strong stress factor stops the autophagy and starts the apoptosis.

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Polyploidy and hybridogenesis: gametogenesis and development of gonads in diploid and triploid water frogs (*Pelophylax esculentus* complex)

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Water frogs are diploid or triploid bisexual hybrids with a special way of gametogenesis, during which one of the parental genomes is eliminated and the resulting gametes are clonal (hybridogenesis) (Graf and Pols Pelaz, 1989). The genome elimination occurrs during a prolonged period of gonial proliferation (Ogielska, 2009). Most probably meiosis starts when one set of chromosomes is properly rejected and the other one is properly reduplicated.

There is no difference in gonad development between the species and hybrids before sexual differentiation, but thereafter gonads in hybrids, both diploid and triploid, display various degrees of delayed development. The cortex of hybrid ovaries is composed of dividing primary oogonia, among which 15–75% degenerate and the onset of meiosis is delayed about 1 year and is prolonged up to 2–3 years. The development and differentiation of testes in hybrids are also delayed and seminiferous tubules contain fewer germ cells. The mitotic activity of primary spermatogonia is high in the hybrids, but most of the resulting germ cells degenerate. Adults are often less fertile and have abnormal gonads. In such testes degeneration of all stages of spermatogenesis is very high, spermatozoa are scanty, and seminiferous tubules are abnormally organized.

The comparison of gonad differentiation between diploid and triploid hybrids revealed that triploids displayed a faster rate of development than diploids. An analysis of genomes transmitted by gametes produced by hybrids suggests that this may be caused by a simpler way of genome elimination before the onset of meiosis: in diploids one of the genome is eliminated and the remaining one is duplicated, whereas in triploids also one genome is elimnated, but two remaining ones (belonging to the same parental species) are ready to enter meiosis and do not need reduplication.

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The ultrastructural studies of the thyroid follicles differentiation in the grass snake *Natrix natrix* L. (Lepidosauria, Serpentes) embryos

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The thyroid gland consists of follicles that are their essential structural and functional units. The morphogenesis of thyroid follicles is a complex phenomenon, involving the proliferation and differentiation of thyrocytes, as well as the coordination of many different cellular activities such as the positioning of cells with respect to one another the setting of inside-outside polarity and the follicular lumen formation. The eggs of the *Natrix* were incubated in the constant temperature at 30°C and the embryos were isolated, starting at eggs lying and finishing at hatching of the first individuals. The age of embryos was calculated using the table of species development (Rupik, 2002). The ultrastructural findings show that: the formation of the junctional complexes between the epithelial cells forming initially cellular cords of the thyroid primordium, and then between cells surrounding the follicular space of developed thyroid follicles was an integral part of lumen formation in grass snake similarly as in all of the vertebrate species. The earliest morphogenesis of thyroid follicles in the grass snake appeared the same as described in chick embryos, but some differences were found in later stages of development. The origin of the thyroid follicular lumen in the grass snake was extracellular as in chick embryos, but the mechanism of its formation was different. The follicular lumen in grass snake embryos was formed by cavitation, because during the thyroid follicles differentiation a population of centrally located cells was cleared by the apoptosis to form the lumen. The differentiation of thyroid follicles accompanies the process of cilogenesis that occurred periodically and new centrioles duplicated via centriolar pathways. The ciliogenesis started in the post-mitotic thyrocytes before their polarisation and follicular lumen formation. It ended approximately halfway through the developmental period when the mitotic cell divisions slowed down and embryonic thyrocytes appeared as evidence of morphological activity. The ciliogenesis restarted before the resting phase when the thyrocytes were polarised and the number of post-mitotic cells increased. This process stopped again shortly before hatching when fully differentiated thyrocytes restarted their activity.

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NOTES: All specimens used in experiment were captured according to Polish legal regulations concerned with wild species protection (Dz.U. nr 2 poz. 11 z 1984 r., Dz.U. nr 114 poz. 492 z 1991 r.). Department of Histology and Embryology obtained approval of Polish Ministry of Environment Protection and Forestry for performing studies on protected species (DOPozgiz-4200/II-88/4189/10/JRO). The grass snake Natrix natrix L. is not included in Washington Convention of 1973, ratified by Poland in 1991(Dz.U. nr 27 poz.112).

3D reconstruction as a visualization method of the glands development in the tongue of the domestic duck

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In the lamina propria of the tongue in birds anterior and posterior lingual glands can be distinguished. In the domestic duck anterior glands are located symmetrically along entoglossal cartilage on the body. Posterior lingual glands are divided into two groups: postero-lateral glands are arranged on the both side of the end of the lingual prominence and postero-medial glands located on the root of the tongue.

In this study the 3D reconstruction method have been used to investigate the development of anterior and posterior lingual glands in the domestic duck. This method allowed to trace changes in glands primordia and characterize if they are formed by branching or elongating.

Anterior lingual glands starts to develop on 13th day of incubation. Glands are formed from the posterior part of the body in the direction of the apex. On the 14th day glands begins to branch. The observation on the 16th day showed that anterior glands are located in groups. Glands in the posterior part of the body have 5–6 branches and glands in the anterior part of the body elongate. Observation conducted on 20th day reveals that openings in the anterior part of the body are arrange perpendicular along long axis of the tongue and in the posterior part are directed ventrally. On the 24th day anterior lingual glands are strongly branched and overlap the cartilage.

The development of postero-lateral lingual glands starts on 12th day of incubation and postero-median glands on the 13th day. Postero-lateral glands develop in the direction of the root and until the 16th day elongate. The postero-medial glands are formed similarly and on the 16th day 3-4 glands openings are observed. On the 20th day the postero-lateral glands begins to branch. On 24th day there are 2 - 3 openings of the postero-lateral glands situate on the both sides of the lingual prominence and two tanks of the mucous on the lateral sides of the root with single openings on the surface of the root. Postero-medial glands on the 24th day are medium branched and 4 – 5 openings are observed on the dorsal surface of the root.

The studies revealed that anterior lingual glands in the domestic duck generally develop by branching and posterior glands by elongating.

The cell death in the midgut epithelium of Myriapoda

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The endodermal part of the digestive system (midgut) of invertebrates is the first barrier for the entire organism against all stress factors which originate from the environment. Therefore midgut cells must have involved some mechanisms which enable the maintenance of homeostasis and protect the organism. The cell death, which leads to the cell destruction, eventually helps in elimination harmful and damaged cells, or even cells which are treated with stress factors.

Some species of myriapods have been chosen for the analysis of the cell death: fast predators which belong to centipedes (Chilopoda): *Scolopendra cingulata*, *Lithobius forficatus*, and slow moving and detritivores millipedes (Diplopoda): *Strongylosoma stigmatosum*, *Archispirostreptus gigas*, *Julus terrestris*. Many studies have been conducted in order to use Myriapoda as bio-indicators in the analyzing of the soil pollution, and they have been mainly connected with the analysis of different organs and tissues which take part in toxic substances accumulation. Therefore we decided to describe processes of the cell death in important bio-indicators.

The midgut epithelium of all analyzed species is composed of three types of cells: the digestive cells, secretory cells and regenerative cells. Digestive cells undergo processes of the cell death, while degeneration has never concerned the secretory cells. Necrosis of digestive cells undergoes sporadic, while apoptosis and autophagy are common processes which enable the cell and the entire midgut epithelium to survive. Apoptosis and necrosis are both known to be involved in the degradation of midgut epithelial cells, while the role of autophagy is mainly implicated in the disintegration of cell's organelles. This study reports on the participation of these three key degenerative processes in the proper functioning of the organism via light, transmission electron microscopy and fluorescent microscopy.

Ovary structure and transovarial transmission of endosymbiotic microorganisms in a primitive scale insect, *Marchalina hellenica* (Insecta, Hemiptera, Coccoidea: Marchalinidae)

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The ovaries of *Marchalina hellenica* have been examined at the ultrastructural level. The ovaries of *M. hellenica* consist of about 200 short telotrophic ovarioles that are radially arranged around the distal part of the lateral oviduct. The individual ovariole is subdivided into three well defined regions: the tropharium (trophic chamber), vitellarium and ovariolar stalk (pedicel). The terminal filament is absent. An analysis of serial sections has shown that tropharia may contain 23–30 individual trophocytes (nurse cells) and single early previtellogenic oocyte (arrested oocyte) or trophocytes only. The arrested oocytes may be located in the anterior region of the tropharium or more centrally among the trophocytes. The localization of the arrested oocytes as well as presence in their cytoplasm of numerous autophagosomes suggest that these cells are not capable of further development. The centre of the tropharium is occupied by a trophic core that is devoid of cells and is connected both with trophocytes and oocytes. Trophocytes communicate with the trophic core by broad cytoplasmic processes, oocytes by nutritive cords. In the vitellaria single oocytes develop. The developing oocytes are surrounded by a single layer of follicular cells. Follicular cells do not undergo diversification into distinct subpopulations. In the cytoplasm of trophocytes and arrested oocytes numerous endosymbiotic bacteria are present. The endosymbionts are rod-shaped and measure about 0.4 μm in diameter and 6 μm in length. The occurrence of endosymbionts both in trophocytes and in arrested oocytes suggests that they infect the ovaries before differentiation of cystocytes into oocytes and trophocytes. In the adult female the endosymbiotic microorganisms are transported via trophic core and nutritive cord to the developing oocyte.

Research problems in the mammalian neo-oogenesis

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The establishment of maximum reserve of the oogenetic cells in early fetal life and the lack of possibility of the multiply in postnatal period are widely known and proved in female mammalian reproductive biology. However, this statement was questioned by Johnson et al. (2004). According to Johnson, there are primordial germ cells in the ovarian envelope (*tunica albuginea*), which are able to undergo the mitotic divisions. Moreover, he postulates that oocytes undergo meiosis and mature in the formed ovarian follicles. This phenomenon was called a neo-oogenesis. The Johnson's publication was the beginning of research on possibilities and efficiency of this process.

Currently there is a lot of scientific evidence for and against the possibilities of the postnatal oocytes formation and the ovarian follicles regeneration in the mammals and, what is more important, in the human. The results of the following research may confirm the neo-oogenesis hypothesis:

1. The morphological tests, in which the histological and histochemical methods as well as the genetic modified primordial germ cells are used.
2. 5-bromo-2-deoxyuridine (BrdU) incorporation and detection in mtDNA anti-BrdU antibody in germ cells located in the ovarian surface epithelium (OSE), which indicates on division activity of examined cells.
3. Cultured of cells derived from OSE gives rise to oocyte-like cells, genetics analysis and transplantation to adult female.
4. Busulphan-induced depletion of the follicle reserve and regeneration de novo of ovarian follicle.
5. Identification of germ cells and oocytes derived from bone marrow and peripheral blood macrophage and follicle regeneration.

The further research, proper interpretation of the results, wide discussion and extensive knowledge of the oogenesis course and control in the mammals and human are required to elucidate this problem (Notarianni, 2011).

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Ovaries organization in clitellate annelids. The present state of knowledge and perspectives

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In clitellate annelids (Annelida, Clitellata) during early gametogenesis syncytial germline cysts are formed. Almost the same pattern of cysts organization has been found in the male and female cyst in all studied so far clitellate species, i.e. each cell in a cyst has only one cytoplasmic bridge connecting it to the central, anuclear cytoplasmic mass, the cytophore (Świątek et al., 2009). The ovaries in Clitellata are composed of germline cysts associated with somatic, follicular cells. Despite broadly similar pattern of cyst architecture, the ovary morphology differs markedly between groups (Urbisz et al., 2010; Świątek et al., 2012). Based on our detailed studies and literature data we have distinguished 7 different types of ovary organization in Clitellata. In our opinion the so-called "oligochaetous" type of ovary is a basal condition for all clitellate annelids, which has been modified during evolution several times and, as a result, the other types of ovaries as "*Enchytraeus*", "*Acanthobdella*", "*Glossiphonia*", "*Piscicola*", "*Erpobdella*" and "*Hirudo*" have appeared. However, for obtaining the full picture of the ovary organization and evolution in Clitellata the other clitellate groups in which the ovary organization is completely unknown, especially basal Capilloventridae, Phreodrilidae and Haplotaxidae and also the rest of Crassiclitellata, Proppapidae and Naidinae should be studied.

Acknowledgements: This work was supported research grant N N303 423636 from the Polish Ministry of Science and Higher Education.

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Formation of cytoplasmic snRNP- rich bodies and Cajal bodies. An in situ and in vivo investigations

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We have used two models hamster ovary cell line (CHO) and larch's microsporocyte cells. The first of these models is characterized by high level of transcription and the second one by natural fluctuations in the level of RNA and proteins metabolism. Using FISH and immungold in situ studies, we have found cytoplasmic bodies and Cajal bodies in both of these cells. Our data indicates that the level of snRNP protein expression and snRNP assembly may be a key trigger for cytoplasmic snRNP-rich bodies and Cajal body formation. In vivo observation has shown that cytoplasmic bodies move within the cytoplasm. Movements include translocations through the cytoplasm, joining of bodies to form larger structures, and dispersion near the nucleus. During our investigation we used microinjection technique and molecular beacon probes for direct visualization of the snRNP in living cells.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Polish Ministry of Science and Higher Education no. N 303799640.

Structure of the female reproductive system in a viviparous earwig *Arixenia esau* (Dermaptera, Arixeniina)

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Viviparity, i.e. the embryo development inside the body of the mother, is relatively rare among insects. In this reproductive mode, the mother's organism provides gas exchange and, what is more important, nourishments for developing embryos. The vast majority of earwigs are oviparous, but in representatives of two parasitic taxa (Arixeniina and Hemimerina) the viviparity was reported. This reproductive strategy probably represents the adaptations for parasitic life conditions, increasing the chances of nymphs in finding the proper host.

Here the analysis of female reproductive system in viviparous earwig *Arixenia esau* is presented. This species has paired ovaries that are composed of 3 ovarioles attached to the lateral oviduct. The ovarioles are relatively short and three elements can be distinguished within an individual ovariole i.e. a terminal filament, germarium and vitellarium.

The oviduct is strongly dilated and contains developing embryos, and therefore can be regarded as the uterus. It should be added however, that the onset of embryogenesis starts within an ovariole.

The uterus wall is thick and deeply folded. It forms prominent invaginations. The TEM analyses showed that in the individual fold, two easily recognizable layers can be distinguished. The outer zone is composed of elongated muscle fibers, between which numerous tracheal tubes are located. The inner layer forms the "uteral ependyma". It is composed of a monolayer of epithelial cells lying on a well developed basal lamina. It should be added that the basal lamina also contains tracheal system branches. The epithelial cells are elongated and are of the characteristic shape. In their basal regions (in the close vicinity to the basal lamina) they are significantly folded and filled with numerous mitochondria and elements of RER. In the middle part of the epithelial cells the nuclei are located. Above the nuclei, numerous vacuoles are present. The most apical parts of these cells are equipped with microvilli that protrude into the uterus lumen. Such a unique structure of epithelial cells suggests their role in the nourishment of the embryos developing inside the uterus.

ACKNOWLEDGMENTS: The study was funded by the National Science Centre (NCN) grant number 2011/01/D/NZ4/00137.

Embryological studies on sterile ecotypes of bolting garlic (*Allium sativum* L.)

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In the sexual method of propagation synchronizing the time when gametophytes reach their maturity is important as it is a factor necessary for fertilization and, in consequence, for producing seeds. In the case of the investigated ecotypes of garlic, there was no period of anthesis, during which it is possible to release the pollen from pollen sacs and to receive the pollen by the receptive pistil. The flowers of the investigated ecotypes of garlic did not reach maturity and were not prepared to begin the pro-gamete phase. Microscopic examination revealed that the examined plants were characterised by lack of a receptive pistil, lack of mechanism releasing the pollen from the microsporangia, and by the fact that that the eventual pollination of flowers did not take place in their case. On the basis of the research conducted it is possible to claim that in Polish ecotypes of garlic both male and female generative lines are sporogenically sterile and that structural sterility occurs in female organs of sexual propagation.

The cranial neural crest in staged human embryos

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The neural crest is a transient population of precursor cells and is characterized by a wide variety of derivatives. The crest is formed at the border between the neural plate and the prospective epidermis and is found in the cranial region before the fusion of the neural folds has occurred. It appears in human embryos at stage 9 (25 days) at the mesencephalon. The pattern of emigration varies according to the species, and the axial level (O'Rahilly and Müller, 2007). The neural crest has cranial, cardiac, and truncal elements.

The aim of present study is to establish the formation of cranial ganglia from the neural crest and trace the relationships of neuromeres to the neural crest.

Contribution of the neural crest cells to the cranial ganglia were investigated in human embryos at developmental stages 12–23 (30–56 days). Serially sectioned embryos were from the collection of Department of Anatomy in Poznań. Sectios were stained according to various methods and impregnated with silver.

Segregation of the neural crest begins at stage 12 and is most advanced during stages 13–15. In embryos at stage 12 (30 days) the trigeminal, facial, glossopharyngeal, and vagal ganglia are discernible and the crest cells of these ganglia migrate into pharyngeal arches. The ganglia are located at the levels of rhombomeres 2 to 7. The hypoglossal crest forms continous column and unites with the hypoglossal cord. At stage 13 (32 days) the nasal crest is formed and the trigeminal ganglion consists of ophthalmic and maxillomandibular divisions. The superior and inferior ganglia of the glossopharyngeal and vagus nerves are formed.

The accessory crest is continous with the spinal crest. During stages 14 (33 days) and 15 (36 days) most of the ganglia formed from the neural crest are observed. The nasal crest reaches the telencephalon and the accessory as well as hypoglossal crests are traversed by nerve fibers. In stages 16 (39 days) and 17 (41 days) vomeronasal and terminalis ganglia develop.

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Gustatory organs in the development of *Rhinella marina* (Linnaeus, 1758)

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At the end of XX century two generations of gustatory organs were documented in the development of anuran and urodelan amphibians, i.e. taste buds in larvae, and taste discs in metamorphosed animals (Żuwała, Jakubowski 1991, 2001). Data presented here constitute a part of research concerning the differentiation of gustatory organs in the development of representatives of the Bufonidae family. The study was carried out on tadpoles of the cane toad, *Rhinella marina*, sampled in various developmental stages (28–45 according to Gosner 1960). Observations were performed under a light microscope and SEM using routine methods. In the studied tadpoles the lining of the oral cavity forms papillae of various shapes (finger-like, palm-like, and bush-like papillae) on the apex of which sensory areas of taste buds can be seen. Non-branching apical parts of finger-like papillae located both on the palate and floor of the oral cavity form the borders of an elliptic field of the lining with numerous taste buds protruding above the surface of the lining. At all studied developmental stages the density of taste buds per 1 mm^2 is higher on the palate than on the oral cavity floor. The diameter of the sensory area of a taste bud ranges between 8–10 μm. The tongue fold forms from the 31st developmental stage in the frontal part of the oral cavity floor. It grows towards the pharynx in subsequent developmental stages of the tadpole. Cell rearrangement on its dorsal surface in stages 35–36 indicates the formation of taste discs. Well-developed sensory fields of tongue taste discs were observed at the 43–44th stages of tadpole development. At the same time the formation of taste discs in the lining of the palate also proceeds. The diameter of the sensory field at the 45th tadpole developmental stage ranges from 8.2 to 36.5 μm, while those located on the palate range between 10.1 to 46 μm.

In conclusion: The developmental stages of two generations of gustatory organs in the studied species closely resemble those observed in Bufo bufo; in the sensory field of a taste disc, as in previously studied species (including Bufo alvarius), there are numerous receptor gustatory cells equipped with abundant short microvilli in their apical parts. Further studies are being conducted using TEM and on one year old individuals.

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POSTERS

The embryonic development of spiders: *Agelena labyrinthica* and *Xerolycosa nemoralis* in variously polluted areas

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Living under environmental pressure caused by chemical stressors requires additional energy resources which should be allocated into defensive mechanisms at the expense of growth or reproduction. Insufficiency of energy may lead to a series of negative responses that can be observed at various stages of egg production and embryonic development which is seldom studied in spiders. In this study we checked how anthropogenic contaminants may affect embryonic development in two spider species: a funnel-web *Agelena labyrinthica* and a wolf spider *Xerolycosa nemoralis* collected at the polluted and the reference sites. We used light microscopy to diagnose histological sections of eggs at different stages of development, and observations of their morphological changes. Computer microtomography was applied to observe changes of the eggs inside untouched and not destroyed cocoons. This is a novel technique, which has never been used previously in that kind of studies. The application of computer tomography enabled us to describe the geometry of eggs inside the cocoons. Moreover, thanks to the 3D visualisation of the scans we analysed the morphology of the embryos to assess their developmental stage.

The study showed that environmental pressure (mostly from heavy metals) had different effect on embryonic development of both spider species. Duration of embryogenesis was longer for the eggs of *X. nemoralis* – 14.5 ± 0.5 days than in *A. labyrinthica* – 11.96 ± 0.6 days (the Mann-Whitney U, p=0,000). Moreover, the embryos of *A. labyrinthica* better survived than these of the wolf spiders. The duration of embryonic and postembryonic stages, and the mortality of embryos differentiated both species and were site-dependent. Embryogenesis was longer in spiders from the polluted site and their mortality was higher than these of the reference area. Results of this study may throw new light on the biology of spiders and on the ability to use computer microtomography in embryological studies.

Ecological aspects of embryonic development of selected carp family species

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Embryonic development of fish species of the carp family (*Cyprinidae*): vimba, *Vimba vimba* (L.), white bream, *Blicca bjöerkna* (L.), ide, *Leuciscus idus* (L.), chub, *Leuciscus cephalus* (L.) and asp, *Aspius aspius* (L.) was observed and analyzed. They are fish species that spawn during spring; their eggs, after being deposed on the water, become adhesive, allowing them to attach themselves to the vegetation, rocks or gravel. Live observation and recording of embryogenesis of the fish species was conducted using: microscope with a cooling system coupled with a digital camera, monitor and VCR. Recorded images were analyzed using image analysis software: Multi Scan v.13.01 and NIS Elements Br.

Study conducted showed that of the fertilized eggs, the smallest size were eggs of the white bream, whose average diameter was 1.53 mm, eggs of the asp, chub and ide were larger by an average of 0.5mm (2.06, 2.10 and 2.13 mm respectively). Duration of embryogenesis, regulated by environment temperature, was the shortest (2–4 days) for white bream and chub, whose embryonic development in the natural environment takes place at the highest temperature of the studied species, 19.0–20.0°C. Development of asp embryos at 8.0–10.0°C, a temperature optimum for this species, took the longest (2025 days) time. Hatched individuals of the studied fish species were characterized by different levels of individual development – they differed significantly in terms of size. The shorter embryogenesis time in chub and bream meant that the embryos attained eye pigmentation stage only after hatching, in the case of vimba, ide and asp, the embryos reached this stage of embryogenesis while inside the chorion; before hatching. White bream is a typical phytophilous species, the remaining species studied were littoral species for this reason, pigment in the eyes of embryos developing inside the eggs, attached to the vegetation, made them conspicuous and exposed to danger. The differences in the structure and construction of egg chorions, egg size, in the course and duration of embryogenesis, between the studied carp-family fish species, are one of the elements of adaptation to ecological conditions under which their embryonic development takes place.

Tetraploids of *Cobitis* occurring naturally may arise from crosses between allotriploid *Cobitis* females and males of the spined loach *Cobitis taenia* Linnaeus, 1758 (Pisces, Cobitidae)

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Some species of the genus *Cobitis* through hybridization processes followed by genome duplication during meiosis produce diploid eggs, which after fertilization develop in triploids. Allotriploids represented almost by females commonly occur naturally in diploid-polyploid *Cobitis* populations distributed in Europe and reproduce mainly via gynogenesis (Boron, 2003). The aim of this study was the experimental induction of gynogenesis of triploid *Cobitis* females from diploid-polyploid population in the Bug River. Oocytes of triploid females were activated by sperm of the spined loach *C. taenia* males from diploid-polyploid population as well as from an exclusively diploid population. All the parental females and males were injected with human chorionic gonadotropin hormone (hCG). All *Cobitis* taxa used in the experiments have been karyologically identified. Chromosomal investigations and/or DNA content by flow cytometry revealed that most of the progeny individuals were triploids with 3n = 74 chromosomes in their cells whereas some other individuals resulted from crossing between 3n *Cobitis* females and 2n *C. taenia* males were tetraploids, with the modal number of 4n = 98 chromosomes. Conclusion: allotriploid *Cobitis* females inhabiting a diploid-polyploid population in the Bug River can produce eggs that develop gynogenetically, without fertilization in triploid progeny and eggs, which are fertilized and develop in tetraploid individuals.

ACKNOWLEDGEMENTS: This work was carried out within the grant no. N N303 068834 supported financially by the Ministry of Science and Higher Education in Poland.

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The cervical part of the sympathetic trunk in human embryos

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The development of the sympathetic nervous system can be divided into three overlapping stages. First, the precursors of sympathetic neurons arise from neural crest cells that migrate ventrally and aggregate adjacent to the dorsal aorta. Second, the cell number is refined when neurotrophic factors determine the number of neurons. The final stage of the sympathetic development is the establishment and maturation of sympathetic connections. In our previous study (Woźniak et al., 2009) it was observed that the primordia of the sympathetic ganglia appear in embryos at stage 13 (32 days) and are located along the dorso-lateral aspects of dorsal aortae at the thoracic levels T4-T9.

Present study was made in human embryos at developmental stages 13–23 (32–56 days). Serially sectioned embryos in three planes were from collection of Department of Anatomy in Poznań. Sections were stained with hematoxylin and eosin, toluidine blue, and impregnated with silver.

In embryos at stage 14 (33 days) the sympathetic ganglia, as aggregates of cells, were observed from the lower cervical (C7) to the sacral region (S1). These aggregates form continuous segmented column along the dorsolateral aspects of dorsal aortae. At stage 15 (36 days) the primordia of the sympathetic ganglia are present in the upper cervical region in close proximity to dorsal aortae. In the cervical region the ganglia form continuous cell column and they are not segmented.

At stage 16 (39 days) the thick, fusiform superior cervical ganglion is well marked. Below this ganglion the cervical sympathetic trunk as a continuous column reaches the stellate ganglion. The ganglia become more compact. The communicating rami from the superior cervical ganglion are observed. The middle and inferior cervical sympathetic ganglia are well marked in embryos at stage 17 (41 days). During the last two weeks of embryonic period the ganglia of the sympathetic trunk acquire definite form.

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Organization of F-actin cytoskeleton in helobial endosperm of *Butomus umbellatus*

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Organization of actin cytoskeleton in helobial endosperm of *Butomus umbellatus* was examined on fixed ovules at various stages of development. Localization of F-actin was detected using monoclonal antibodies and visualized by immunofluorescent microscopy. In helobial endosperm the first division of primary endosperm cell is asymmetric. One of the daughter cells (smaller, called chalazal chamber – CC) remains uninucleate while the other (larger, called micropylar chamber – MC) undergoes a series of free nuclear divisions before cellularizing. A large multinucleate syncytium in MC results from repeated mitoses without cytokinesis. At this phase of MC development F-actin is visible in the cytoplasm as an irregular network which becomes denser around elongated free nuclei. In a few cases microfilaments are longitudinally aligned between nuclei. MC cellularization is gradual and centripetal, starting from the micropylar pole of the embryo-sac around the suspensor basal cell and extending peripherally toward the chalazal chamber. The course of this process is similar to cellularization described in nuclear endosperm. After cellularization which has been accompanied with reorganization of actin cytoskeleton, microfilaments are not so numerous, become increasingly concentrated around spherical nuclei and also distributed along transvacuolar strands of MC. The chalazal chamber remains uninucleate. Actin cytoskeleton in the CC is usually irregular but in some cases showed longitudinal arrangement, particularly in chalazal-micropylar axis. Such positioning of the microfilaments can be seen as another proof of the functioning this cell as structure which plays an important role in the development of young seed transferring nutrients from chalazal nucellar cells to micropylar chamber. Moreover the regular formation of these actin arrays suggests their fundamental role in the movement of organelles inside such a large cell. High developed actin cytoskeleton is also observed around huge, polyploid nucleus – this may indicate the involvement of microfilaments in the intracellular distribution of nuclear products and positioning of this structure.

The role of hemocytes in the proper functioning of the midgut epithelium of centipedes (Myriapoda, Chilopoda)

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Hemocytes of centipedes form a kind of "immune system" which takes part in the protection of the organism against pathogens such as bacteria, viruses or fungi. Hemocytes are also involved in processes of self-renewal after cuticle and epidermis injures, or in encapsulation. They cause the phagocytosis of smaller pathogens and the encapsulation of larger ones. In Arthropoda many kinds of hemocytes have been described. However, only three types of these cells (prohemocytes, plasmatocytes and granulocytes) commonly occur in the body of all centipedes (Xylander, 2009).

We examined the midgut of two species of Chilopoda – *Lithobius forficatus* and *Scolopendra cingulata*, because it is the first organ of the body, that is exposed to the toxic substances or activity of pathogens. All hemocytes in analyzed species are characterized by ameboid shape, irregular nucleus which is located in the central part of cell and cytoplasm rich in electron-dense granules which indicates that they are granulocytes.

In both analyzed species hemocytes were observed between visceral muscles and beneath the basal lamina. Hemocytes between the digestive cells in the midgut epithelium appeared only in those specimens of *L. forficatus* that were infected with pathogens (rickettsia-like microorganisms were observed in about 10% of the specimens of *L. forficatus*). In case of *S. cingulata* hemocytes occurred between digestive cells of the midgut epithelium in each of analyzed specimens (the cytoplasm of all digestive cells possesses numerous endosymbionts, while the midgut is infected with protozoan). The presence of hemocytes between midgut digestive cells in the specimens that had been infected with pathogens and their ameboid shape, suggest that they are able to move through the basal lamina. The midgut epithelia of analyzed species are covered with the peritrophic membrane, which plays a role of the barrier against pathogens. However many microorganisms are capable of infecting the midgut epithelium despite the presence of a peritrophic membrane. When the pathogens infect the midgut epithelium of centipedes, hemocytes protect the entire organism against their migration into the body cavity.

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Embryo-suspensor in *Sedum reflexum* L.

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The suspensor is a terminally differentiated embryonic region that anchors the embryo-proper to the surrounding maternal tissue and serves as a conduit for nutrients and growth regulators supporting embryo proper development (Yeung and Meinke, 1993; Kozieradzka-Kiszkurno et al., 2011a,b). The development of the suspensor (consisting of a basal cell and a few chalazal cells) in *Sedum reflexum* L. was investigated using cytochemical methods, light and electron microscopy. *Sedum* undergoes the Caryophyllad type of embryonic development. The basal cell produces haustorial branches invading the micropyle and adjacent tissues. The micropylar part of the suspensor basal cell and the micropylar haustorium are covered with wall ingrowths typical of transfer cells. Cytochemical results on the composition and distribution of macromolecules (proteins, insoluble polysaccharides and lipids) at various stages of the development of the embryo proper and suspensor, and analysis of the suspensor ultrastructure in *S. reflexum* show that the basal cell is a site of intense metabolic activity.

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Using of the biodiversity of Poaceae family species in improving of wheat *Triticum aestivum* L.

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

The experimental material consisted of 28 lines of winter wheat obtained using the interspecific and intergeneric hybridization of *T. aestivum* L. with alien species *T. durum* Desf., *T. timopheevii* Zhuk., *Lolium perenne* L. and *Aegilops speltoides* Taush. Among them, 15 lines were developed from the cross-combination with tetraploid species (*AABB*) *T. durum* Desf., 4 lines from the combination with other tetraploid species of different genome composition (AAGG) *T. timopheevii* Zhuk., 4 lines from cross with *L. perenne* L. and 5 lines were the double hybrids (three-generic) derived with two related species, *T. durum* Desf. (AABB) and *Ae. speltoides* Taush (*BB*).

Anther culture method was used for obtaining DH lines from these interspecific and intergeneric hybrids. In in vitro culture 124 green plants were regenerated. The method of cluster analysis grouped hybrids in terms of comprehensive general similarity of the studied traits.

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Temperature-dependence of cells size in *Helix aspersa* snails

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Low temperatures retard growth rate of cold-blooded animals, but ectotherms often reach maturation later and get larger size as adults in cold. Although this pattern, called the "temperature-size rule" (TSR), is observed in many ectotherms, some of them do not follow it. In some ectotherms, temperature-mediated changes in body size can be associated with changes in cell size, but it is unknown how commonly this phenomenon occurs in nature and what is its causal relationship with TSR. Here we report results of our study that compared cell size and nucleus size of two subspecies of *Helix aspersa* snails (*H.a. aspersa versus H.a. maxima*) that were developed at 15°C and 20°C. Bodies of 47 adult snails were preserved in Bouin's solution, dehydrated in graded ethanols and butyl alcohol, cleared in chloroform and embedded in paraffin wax. Serial sections, 5 μm thick, from three kinds of somatic tissues: external epithelium of body, locomotor muscles and gland cells of hepatopancreas were made with a rotary microtome. Slides were stained with haematoxylin or haematoxylin and counterstained with eosin Y, cleared in xylene and mounted in DPX. Tissues were photographed with a digital camera under a light microscope, and cells and their nuclei were measured.

ACKNOWLEDGEMENTS: The work was supported by a programme "DS for development of young researchers and PhD students, K/DSC/000171" of the Faculty of Biology and Earth Sciences, Jagiellonian University.

The level of metallothionein BnMT1-BnMT3 transcripts in seeds of *Brassica napus* L.

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Metallothioneins (MT) are low-molecular-weight proteins, which owing to the presence of many cysteine residues are capable to covalent binding of heavy metal ions. Plant MTs are involved in some important developmental processes, such as embryogenesis, root development, pollen germination and seed germination (Hassinen 2011).

Brassica napus L. methallotionein (*BnMT1-BnMT3*) transcript level was analysed by sqRT-PCR method. The expression of studied genes varied in dry and germinated seeds. *BnMT1* and *BnMT2* transcripts were found in all the studied samples (dry seeds, seeds germinating 1–48 hrs in the water, and five days old seedlings). The rape *BnMT2* transcript was subject to the smallest fluctuations reaching the lowest level in the 36 hr of the germination and the highest in 5 days old seedling. The *BnMT1* and *BnMT3* expression occurs at very different levels during the germination. The highest level of the *BnMT1* mRNA was observed after 6 hrs of imbibition and was 10-fold higher then in dry or shortly soaked seeds. The *BnMT3* expression occurs after 24 hrs of the imbibition and during another 12 hrs reaches high level lasting for the next 3.5 days. The functional analysis showed that *BnMT2* transformed into the bacteria in expression vector improved the *Escherichia coli* cells growth in the media with copper and lead ions. In turn the bacteria bearing pET21a-*BnMT3* construct grew better than control bacteria in the presence of cadmium ions.

The presence of many methalothionein isoforms in the dry and germinating seeds and their diverse expression pattern indicates various MT functions played in the plant cells. Their role in seed development was previously suggested by our in silico analysis showing presence of RY element of seed specific expression in one of *Arabidopsis thaliana* MT promoters.

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Higher Education of Poland, the project no: N N310 285337.

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The involvement of endogenous auxins in androgenesis of *Brassica napus*

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Haploids/doubled haploids (DHs) provide valuable material for breeding, basic research and biotechnology. Nowadays, technologies for DHs production are adapted for nearly 300 crop species. However, some of these protocols suffered from unsatisfactory efficiency, which limited its utilization on a large scale. To the most important factors influencing DH production efficiency belong: growth conditions of the donor plants, their physiological status, the stage of pollen development and culture conditions. All of them influence the endogenous level of plant hormones (GR). Among them auxins have been identified as involved in androgenesis induction. Better understanding of auxins role and the mechanism of its action in the process of microspore embryogenesis could bring a progress in DH technology utilization. *Brassica napus* microspore suspensions is considered to be a perfect model for such study.

In the study, two rape-seed genotypes (cv 'Topas' and line DH 4079) with different embryogenic capability were used. Endogenous auxins (IAA, IBA) were extracted and purified by common chromatographic technique from leaves, flower buds and microspores isolated from plants growing at different temperature conditions (18°C/10°C).

In general, the level of various auxin forms depends significantly on the sample source and temperature growth conditions. In leaves, IAA level at 18°C exceeded 55-fold IAA concentration at 10°C. Conversely, plant transfer from 18°C to 10°C was accompanied by 5-fold increase in IBA level. It was also revealed that IBA content in leaf extract was much lower (ca.100-fold) when compared to IAA. On the contrary, auxin derivate that prevailed in isolated microspores was IBA. To our knowledge, this is the first report showing IBA as a main auxin form in microspores.

Our findings show possible importance of IAA/IBA ratio in establishment of auxin homeostasis in donor plants as well as in immature pollen grains used for microspore embryogenesis. However, to explain this phenomenon, further investigations are needed.

ACKNOWLEDGEMENTS: This work was supported by agreement with the Polish Academy of Sciences (PAS) – The Research Foundation-Flanders (FWO) in frame of joint polish-belgish project: "Auxin as a trigger in double haploids (DHs) production of oilseed rape" and the national project 2011/01/D/NZ9/02547.

Development of the digestive tract in stage IV larvae of *Contracaecum rudolphii* Hartwich, 1964 (Nematoda: Anisakidae)

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The structure of the digestive tract of the genus *Contracaecum* is a highly important in taxonomy. In adult individuals of *C. rudolphii* Hartwich, 1964, the ratio of the length of anterior intestinal caecum to the length of the posterior ventricular appendix accounts for 3:1 (Baruš et al., 1978). Rudiments of both outgrowths are visible as early as in the first larval stage. In the second and third stage, the ventricular appendix is already clearly visible, the intestinal caecum is much shorter.

The objective of this study was to conduct observations of the development of the digestive system in the fourth stage larvae.

The larvae isolated from cormorants (*Phalacrocorax carbo*) were fixed in the 70% ethanol, cleared in lactophenol and identified according to keys by Baruš et al. (1978) and based on the descriptions given in Abollo et al. (2001), Amato et al. (2006), Kanarek and Bohdanowicz (2009). Measurements and photos of the larvae were taken under an Olympus microscope with the aid of a computer software for image analysis Multiscan v.4.2.

In the larvae, particular elements of the gastrointestinal tract (esophagus, stomach, intestine) ventricular appendix and intestinal caecum were clearly visible. The intestinal caecum to ventricular appendix length ratio was found to increase with growth of *C. rudolphii* fourth stage larvae from 1:1 to 1.5:1.

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Selected aspects of flowering biology of *Primula farinosa* L. from the only Polish locality

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Primula farinosa L. from *Primulaceae* family is protected, rare and critically endangered species of Polish flora. Scattered nine lowland localities are now historical and the only population in Jaworki in south Poland is the subject of the national monitoring projects (Kaźmierczakowa, 2001). The population has been decreasing rapidly for the last forty years and now it consists of about 150 flowering individuals. The reasons of this can be complex and one of possible can involve problems of generative propagation. The research to examine chosen aspects of it was undertaken in 2011 season. In the course of field studies the number of flowers per inflorescence and the effectiveness of fruit setting were assessed. The flowers morphology was described based on the biometric measurements in order to determine the presence the long-style and short-style flowers. Pollen viability was assessed with the Alexander dye as well as in the direct method – germination on the medium, embryo viability was checked in tetrazoline test.

The number of flowers per inflorescence ranged from 3 – 21, an average 9.7. The average number of capsules per inflorescence was 7.9 which means that the effectiveness of fruit setting was 80.1%. Most (86%) of examined flowers had typical for the species corolla (five petals) the remaining had three, six or seven elements. The height of the pistil ranging from 2.6 to 5.0 mm, as well as the distance of anthers from the receptacle ranging from 2.6 to 5.5 mm proof the presence of long-style and short-style flowers. The viability of pollen in both method was 100% and seed (embryo) viability just after fruit opening was 95%.

ACKNOWLEDGEMENTS: This work was supported by the Stary Sącz Local Office of the Regional Directorate for Environmental Protection in Kraków within the project B/030/11/28 "Active and conservation protection of the bird's-eye primrose on the only position in Poland in Beskid Sądecki within the Natura 2000 area".

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Somatic embryogenesis in leaf- and hypocotyl-derived protoplast cultures of carrot

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In carrot, usually cell suspension cultures and petioles are used as donors for protoplast isolation. Despite the fact, that suspension cultures are an excellent protoplast source due to the high embryogenic ability, their establishment and maintenance is laborious and time consuming, usually requiring several weeks in addition to the time needed for callus induction. In turn, the protocol using petioles is not efficient enough when plant regeneration is obtained through indirect somatic embryogenesis (Dirks et al., 1996). Thus, the aim of the present work was to establish a fast and easy system of plant regeneration via direct somatic embryogenesis from carrot protoplasts, which would be applicable in research e.g. on protoplast fusion, in vitro selection or genetic transformation.

Protoplasts of seven carrot accessions were isolated from leaves with petioles of 3-4 week-old plantlets and from hypocotyls of 2 week-old seedlings. Tissue digestion preceded with preplasmolysis treatment was performed overnight in enzyme mixture supplemented with 1% cellulase Onozuka R-10 and 0.1% pectolyase Y-23. A modified thin alginate layer technique was applied for the protoplast culture. A simplified Kao and Michayluk medium containing 2,4-D and zeatin was used to induce protoplast divisions and somatic embryogenesis while medium based on MS macro- and micro-elements without growth regulators was required for complete plant regeneration (Grzebelus et al., 2012).

The protoplast isolation efficiency reached on average 3 x 106 and 106 protoplasts per g of leaf and hypocotyl tissue, respectively. Direct somatic embryogenesis occurred during cultivation of both leaf- and hypocotyl-derived protoplasts for all accessions used. Morphologically normal plants were produced at very high efficiency within two months after initiation of the protoplast culture. Ninety three percent of the regenerated plants were diploids. Pollen viability and seed set after self-pollination were similar to those of plants obtained from seeds.

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Early development of the neural arches in staged human embryos (6 and 7 weeks)

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The vertebrae develop from somites and in their formation two structures are important: 1) the perinotochordal sheath, and 2) the loose and dense zones of the sclerotomes. The perinotochordal sheath and loose zones of the sclerotomes form the vertebral centrum which forms most of the vertebral body. The dense zone of the sclerotome develops into the neural arch and rib. The centrum and the neural arch ossify from the primary centers and the body of adult vertebra consists of the centrum and a part of the neural arch which is more extensive than the vertebral arch.

Investigations were performed on 28 human embryos at developmental stages 16–20 (39–49 days). Embryos were from collection of Department of Anatomy in Poznań and were serially sectioned in three planes. Sections were stained with routine histological methods and impregnated with silver.

In embryos at stage 16 (39 days) and 17 (41 days) the upper two cervical vertebrae are not separated and the developing dens is fused with the atlas. The well developed vertebral centra are longest in the cervical region. The notochord is narrower in the centra and becomes segmented. The perinotochordal sheath is distinct and the beginning of chondrification is noted. The neural arches surround half of the spinal cord. The transverse processes are discernible and in the ribs the head, neck, and tubercle are marked.

During stages 18 (44 days) and 19 (46 days) in the atlas differentiates anterior arch located anteriorly to the dens. The vertebral column as a whole is less curved. Within the intervertebral disc the anulus fibrosus is forming. The articular processes and vertebral arches chondrify.

In embryos at stage 20 (49 days) the vertebral bodies are of different size and shape in particular parts of the vertebral column. The curvatures of the vertebral column may be observed. The growing neural arches extend above the intermediate gray matter of the spinal cord.

Immunocytochemical studies of F-actin in the generative cell of pollen grains of *Convallaria majalis* L.

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This study shows evidence of F-actin in mature pollen using indirect epifluorescence microscopy. The cytoskeleton has a particularly important role in the plant cell cycle. Microfilaments (MFs) arrays are present during interphase and appear in the phragmoplast when microspore divides into generative and vegetative cells. To date, it is believed that at this stage of male gametophyte development in angiosperms, F-actin is no longer observed, and only depolymerised, G-actin is present in generative cell (GC). Many authors show F-actin in the cytoplasm of vegetative cells, and indicate its absence in both the generative cell, as well as in sperm cells. However, some researchers have provided evidence of MFs presence in *Ledebouria socialis*, but only at the level of transmission electron microscopy. Immunogold labeled actin was observed near microtubule bundles occurring there (Hess et al., 1995). Also two papers by Zhu et al. (1980) and Russel and Cass (1981) reported MFs in the sperm of wheat and *Plumbago*, respectively, but the results are still not convincing. Mature pollen grains of *Convallaria majalis* show unique actin arrays in GC. After asymmetrical mitosis pollen grain contains two cells with very different sizes, nuclear morphologies and developmental fates. Both cells have dense microtubular and microfilament cages surrounding their nuclei. Following cytokinesis, F-actin and α-tubulin ring encloses the generative nucleus and both cytoskeletal networks are observed in whole generative cell. Such situation is very common for microtubules observation but not for MFs bundles.

ACKNOWLEDGEMENTS: This work was financially supported by internal grant 'Young Scientist' 538-L160-0778-1 from University of Gdansk.

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Analysis of self-incompatibility in cabbage (*Brassica oleracea* L.)

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Cabbage hybrid breeding in Poland is based on the use of self-incompatible lines. Our research was primarily aimed at identification of S-haplotypes which were present in Polish breeding materials. For this purpose we used four different methods. The biological method was based on the number of seeds produced upon self-pollination. In the fluorescence method germination of pollen grains and growth of the pollen tubes was examined under florescence microscope. In addition to that two molecular methods were exploited – PCR-RFLP and DNA sequencing. The PCR-RFLP analysis was performed on the amplified fragments of two genes – *SLG* and *SRK*. The former is coding for *S*-locus glycoprotein and the latter – *S*-locus receptor kinase. The sequence analysis was performed on the amplified fragments of the *SRK* gene. To facilitate the sequence comparison a dedicated computer program was developed. In all these investigations the S-haplotype tester lines obtained from the Vegetable Gene Bank in Wellesbourne (UK) were used as a reference. In the result of this examinations in the Polish breeding lines of cabbage the following *S*-haplotypes were found: S^2, S^7, S^{14}, S^{15}, S^{20}, S^{28}, S^{38}, S^{50}, S^{51} and S^{55}.

In modern breeding of hybrid cabbage the control of pollination is accomplished with the use of cytoplasmic male-sterility (CMS) which requires the maintainer lines to be self-compatible. Therefore, in another set of experiments we searched for molecular indicators of self-compatibility. With the use of RT-PCR we compared self-compatible and self-incompatible cabbage lines with respect to accumulation of the *SLG* and *SRK* transcripts. We found that self-compatibility was accompanied with decreased accumulation of the *SRK* mRNA.

ACKNOWLEDGEMENTS: This work was supported by Polish Ministry of Agriculture and Rural Developmant (grant No. HOR hn 801-22/11, No. 86, 105)

Correlation between cytoplasmic snRNP-rich bodies and *de novo* formation of Cajal bodies

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In eukaryotic nuclei there are dynamic structures, involved in splicing and snRNPs maturation called Cajal bodies (CBs). One characteristic trait of CBs is the dynamic changes in their size and number in different species, tissues or cells and can be related to the metabolic activity of a cell. The increasing number of CBs could be the result of their *de novo* formation. This process is correlated with snRNP biogenesis which includes a sequence of events that occur in both the nucleus and cytoplasm. Despite the wealth of biochemical information, little is known about the spatial organization of snRNPs in the cytoplasm.

We have used diplotene larch microsporocytes as a model system to examine the organization of cellular snRNPs. This model is characterized by strong fluctuations in the number of CB and natural changes in the level of RNA metabolism. In the cytoplasm of the larch microsporocytes, a cyclical occurrence of bodies containing snRNA and Sm proteins was observed during diplotene. We have investigated the hypothesis that occurrence of cytoplasmic snRNP bodies is responsible for managing the formation of Cajal bodies.

ACKNOWLEDGEMENTS: This work was supported by a grant from the Polish Ministry of Science and Higher Education no. N 303799640.

Growth regulator-dependent differences in somatic embryo morphology, nuclear DNA content and ability to plant regeneration in culture of *Trifolium nigrescens* (Viv.)

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An approach combining the morphological profiling and flow cytometric analysis of somatic embryogenesis (SE) from zygotic embryos of *Trifolium nigrescens* Viv. was performed to assess the relation between the nuclear DNA content, morphology and anatomy of somatic embryos and their ability to establish plants (Konieczny et al., 2012). SE was induced on MS medium containing 4 mg l^{-1} 2,4-D and 2 mg l^{-1} 2iP (MSD) or 0.5 mg l^{-1} NAA and 2 mg l^{-1} 2iP (MSN) either directly from hypocotyl or via callus according to the time of culture. Beside the embryos of zygotic-like morphology, abnormal phenotypes were observed on both media, but the rate and types of abnormalities were significantly higher in callus culture. Among abnormal somatic embryos, classified according to morphological traits as horn-shaped, polycotyledonary and fused embryoids, the horn-shaped were the most frequent. Histological observations revealed that unlike the somatic embryos of typical morphology, the horn-shaped embryoids never developed shoot and root meristems and their cells underwent parenchymatization. Only somatic embryos displaying zygotic-like morphology regenerated into plants. The genome size of zygotic embryos, abnormal and zygotic-like somatic embryos developed directly or via callus on both media was compared using flow cytometry. The nuclear DNA content of the species was about 0.9 pg/2C. No significant differences in 2C-values between zygotic embryos and somatic embryos obtained on medium with NAA and 2iP were detected. Horn-shaped embryos derived from MSD medium possessed increased genome size; among the zygotic-like embryos obtained indirectly on this medium the individuals with species-specific as well as increased DNA content were observed. The results indicate physiological rather than genetic background of phenotypic abnormalities in cultured material. The mean 2C DNA content of all regenerants was characteristic for the species, suggesting that only diploid embryos could convert into plantlets.

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Early ontogenesis of the sand smelt *Atherina boyeri* Risso, 1810

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The objective of this study was to investigate the characteristics of embryonic development of *Atherina boyeri* of family atherinidae, occurring in the coastal marine zone waters of the subtropical zone between 53°N–20°N, 18°W–42°E.

Spawners were harvested from the waters of the Kaštela Bay. After fertilization, the eggs were incubated under optimal oxygen and thermal conditions (temp.=21.0±0.2°C, salinity 37.8PSU). Embryonic development were observed and recorded by means of: a microscope with a camera, monitor, VCR and NisElements computer software.

Atherina boyeri egg chorions were thin, equipped with long, sticky appendages for attaching eggs to underwater vegetation, smooth outside surface with few pores, while the inner side had lots of craters. The diameter of hydrated eggs was 1.22±0.04 mm, and the yolk sphere 0.96±0.03 mm, hence giving volume of hydrated eggs as 1.35±0.10 mm^3, and yolk sphere 0.96±0,06 mm^3.

45 minute (16H°) after fertilization, perivitelline space, which occupied about 20% of the volume of eggs, emerged. After 50 minutes (18H°), ectoplasm agglomerate on the animal pole, forming a fertilization cone. After 65 minutes (23H°), first division of the embryonic disc – two blastomeres already formed, the embryo reached morula stage after 13.5 hours from the moment of activation (in 290H°).

Peristalsis occurred during gastrulation. At the 39th hour of development (838H°), the heart slowly started its work, at a rate of 40 beats/min, but 48 hours later it had reached a rate of 130–140 beats per minute. First somatic contractions of the embryo was observed at the 52nd hour (1118H°) of the embryonic development. After one day, the range of body movements had expanded, and their number had increased to about 3–4 movements/minute. At the 120th hour of development (2580H°), the embryo started to slowly move the pectoral fins. Towards the end of embryonic development, the embryo started to move its mouth. Pigment in the eyes appeared after 45 hours of development (968H°), and appeared on the body after 70 hours from the moment of fertilization of eggs (1505H°).

After more than 200 hours (4300H?) from fertilization, the embryos started to hatch. Hatched individuals measured, on average, 4.29±0.04 mm and had yolk sac of volume 0.51±0.02 mm^3. The larvae were slender, with melanophores concentrated mainly on the sides of the body and the head.

Ultrastructural studies on bacterial endosymbionts in psyllids (Insecta, Hemiptera: Psylloidea)

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The ultrastructure, distribution and transovarial transmission of endosymbiotic microorganisms in six species of psyllids, *Psylla alni, Spanioneura buxi, Cacopsylla ulmi, Psylla mali, Rinocola aceris, Cacopsylla crataegi* have been studied. All of them belong to the family Psyllidae. Ultrastructural studies have shown that in all the examined species two kinds of morphologically distinct endosymbiotic bacteria occur. The more numerous bacteria of irregular shape that occur in all the examined species represent the primary endosymbionts (P-symbionts) of psyllids. Besides the primary endosymbionts, examined psyllids contain also less numerous secondary endosymbionts (S-symbionts). The latter are characterized by diverse shapes and sizes in each species. The endosymbionts tightly fill the cytoplasm of large, polyploid cells termed bacteriocytes. The bacteriocytes form large organs termed bacteriomes which are located in the closest neighborhood of the ovaries. As a rule, in the body of investigated species two bacteriomes are present. Each of them is composed of a large multinucleate syncytium (containing secondary endosymbionts) and individual, uninucleate bacteriocytes (containing primary endosymbionts).

Both primary and secondary endosymbionts are transmitted from the mother to the offspring vertically (= transovarially). The infection of the oocyte takes place during the advanced vitellogenesis. The endosymbionts are released from the bacteriocyte cytoplasm and start to migrate towards ovaries. When they reach the oocyte surface they transverse the follicular cells and gather in the perivitelline space (i.e. space between the oocyte and follicular epithelium). The endosymbionts accumulate in a deep invagination of the oolemma at the posterior pole of the oocyte and form a characteristic "symbiont ball". Next, the oocyte becomes covered by egg envelopes. The obtained results indicate that the psyllids belonging to the family Psylidae are characterized by the same mode of the transovarial transmission of endosymbiotic microorganisms.

The micromorphological and histochemical studies of *Cirrhopetalum weberi* (Ames) Senghas (Bulbophyllinae, Orchidaceae)

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Cirrhopetalum weberi is taxonomically situated in the typical section of *Cirrhopetalum sensu* Garay et al. (1994). The species grows as epiphyte or lithophytes in Phillipines. Flowers are arranged in the umbel, characteristically for the imitation of radial flowers, which produce nectar. The two lateral sepals joined together play a role as landing platform for insects. They are built by flat cells without any noticeable secreted substances. The dorsal sepal and petals are covered by papillae with an undulated epidermis. At the apex the elevated groups of cells with stomata are present. The characteristic multicellular appendixes are visible at the margins. Cross sections of papillae on petals and dorsal sepal indicate the presence of starch grains, proteins, larger cells with raphides. The lip is curved, fleshy, smallest in regard to other tepals. Cross sections of lip cells show single layer of epidermis rich of proteins and polysaccharides, minor starch grains, lipid droplets, some raphides. These results of epidermises of lip, petals and dorsal sepal may indicate the secretive activity (according to the previous results: Kowalkowska, 2009; Kowalkowska et al., 2010; 2012). The ultrastructural research will be held.

ACKNOWLEDGEMENTS: This work was supported by the University of Gdansk (DS./L150-4-0128-1) and by the National Science Centre in Poland (N N303 580439). We are grateful for the samples send by Anton Sieder from Botanischer Garten der Universität Wien. The voucher number is WU-324/92.

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Morphology and structure of plastids in embryo-suspensor among Crassulaceae

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From a morphological and structural point of view, plastids in very physiological active organs, which serve transporting and haustorial (nutritional) functions, are especially interesting. One such organ is the angiosperm suspensor, which performs these functions for the developing embryo (Kozieradzka-Kiszkurno and Bohdanowicz, 2010; Kozieradzka-Kiszkurno et al., 2011). The plastids in the suspensor cells of Crassulaceae are distinctive and quite different from plastids found in the rest of the embryo sac similar to other angiosperm. Diversity within suspensor plastids was evident in this family (representatives of the four genera). Unique features of suspensor plastids (e.g. the occurrence of numerous small tubules and electron-translucent and electron-dense inclusions) are presumed to synthesize specific nutrients and/or substances which control embryonic development. A decreasing gradation in the complexity of plastids from the basal cell to the chalazal suspensor cells and the embryo proper was noted in all of the species in this family. The results of this study are an indication of a high degree of diversification and specialization in plastids during plant embryogenesis in Crassulaceae. It is believed that suspensor's plastids may play an important role in the metabolism of the suspensor and thus influence the development of the embryo proper, and that they are also involved in the autolysis of the embryo suspensor – the programmed cell death of this organ (Schwartz et al., 1997).

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The magnetic field as a factor in water management in the early stages of development of the European white-fish (*Coregonus lavaretus* L.)

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The Earth's magnetic field is a constant element of the biosphere affecting the life functions and behavior of organisms. Technological progress has meant there is an increasing number of sources of magnetic field, which may significantly affect important physiological processes, such as the permeability of limiting membranes, on which proper functioning of water management of the egg cells depend (including flow of ions and metabolites) that are particularly sensitive to any environmental changes. The aim of the study was to investigate the effect of generated magnetic field on the process of water absorption by the European white fish eggs.

Fertilized European white fish eggs that were placed in a constant magnetic field of intensity 10mT, constituted the study material. Eggs in the control setting were under natural magnetic field. The developing perivitelline space was observed and documented using a Nikon Eclipse TE2000S microscope with an integrated digital camera. The images of eggs were recorded 0.5h, 1h, 1.5h, 2h and 24h after fertilization, then analyzed using specialized computer software, NIS-Elements Br.

It was found that incubation of fertilized eggs under a constant magnetic field of intensity 10 mT caused an increase in the volume of the perivitelline space as well as the whole egg. Half an hour after fertilization, perivitelline space as a percentage of the volume of whole egg, in the sample exposed to magnetic field, was 34.02%, but 33.7% in control setting. After 1h, 1.5h, 2h and 24h, perivitelline spaces in the eggs under magnetic field assumed the following values: 35.73%, 35.73%, 36.22% and 38.08% respectively. While in the control setting the values were: 33.98%, 33.2%, 35.24%, 36.44%.

Results obtained during the study have confirmed that exposure to constant magnetic field increases the permeability of egg chorions, resulting in the increase of not just perivitelline space alone, but also of the size of whole egg.

Identification of QTLs controlling ABA accumulation in triticale (× *Triticosecale* Wittm.) anthers in response to androgenesis-inducing stress treatment

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Abscisic acid (ABA) is the plant hormone which plays a key role in plant development and adaptation to environmental stresses. Among others, ABA regulates embryogenesis process and its role in microspore switch toward androgenic development has been suggested. It was proved that ABA accumulation is regulated by several genes and inherited in a quantitative manner.

The analysis was conducted on 'Saka 3006'× 'Modus'mapping population of winter hexaploid triticale (× *Triticosecale* Wittm.) that consisted of 72 doubled-haploid lines. ABA content was measured in anthers collected from freshly cut tillers (control) and from tillers after androgenesis-inducing treatment (3 weeks at 4°C) by indirect enzyme-linked immunosorbent assay (ELISA). QTL analysis (CIM, Kruskal-Wallis test) were performed with Windows QTL Cartographer and MapQTL 5.0 package.

The CIM analysis revealed one QTL on chromosome 5A for control plants and 4 QTLs on chromosomes 2A (2QTLs), 1B and 5R controlling accumulation of ABA in response to low temperature. Kruskal-Wallis test revealed additional markers on chromosomes: 4A, 5A and 2RS.3R (control) and 3A, 3B, 5B and 2RS.3R (cold treated plants).

ACKNOWLEDGEMENTS: The research was conducted in the frame of the COST Action FA0604 'Triticeae genomics for the advancement of essential European crops (TritiGen)' and supported by the National Science Centre (NCN) grant number 548/N-COST/2009/0.

A comparison of selected behaviors observed in breeding with those described in mudskippers in natural habitats in reference to reproductive biology of *Periophthamus barbarus* Linnaeus, 1766

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Periophthalmus barbarus is one of 35 amphibian species from Oxudercinae subfamily. In nature this species inhabits tidal zone of the west coast of Central Africa. Breeding biology of mudskippers is not well examined due to the difficulties with habitats availability. Many aspects of this issue remains a mystery. Breeding of this species in captivity is possible but it has not been reproduced to date. That's why all the specimens are imported from natural habitats. In order to learn more of mudskippers reproductive biology, a breeding establishing natural conditions was constructed (tides, the daily change of light intensity, the daily change in temperature and humidity, the daily change in water oxygenation). Breeding was equipped with a monitoring system that allows to record video from three cameras on a continuous basis. With such solutions some behaviors have been observed what is associated with reproduction behaviors reported previously in individuals of other species in the wild (Ishimatsu et al. 2007). The most important was digging an underground passage and defense of territory.

ACKNOWLEDGEMENTS: This work was supported by grant: 5643/B/P01/2010/39.

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Embryological studies on *Biscutella leavigata* L. (Brassicaceae) from soils with elevated heavy metal levels

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Biscutella leavigata L. is a glacial relict found only in the Tatra Mountains and in isolated locations in the region of calamine waste heaps. Although there have been various studies concerning the species (e.g. Godzik, 1991; Mesjasz-Przybyłowicz et al., 2001; Orłowska et al., 2002), nothing is known about the embryological processes in plants from polluted sites. The studies were conducted in order to determine the environmental stress conditions on the reproduction of *B. leavigata*. The examined specimens originated from the old heap in Bolesław, and from two control populations: the Jaworzynka Valley and the Mountain Botanic Garden in Zakopane.

Observations of flower buds showed that in ca 8% of young ovules of specimens both from Bolesław and control sites 2 embryo sacs were found.

Developmental disturbances and necrotic processes during female gametophyte development occurred in 13% of flowers from Bolesław. They mostly included necrosis of egg apparatus and whole embryo sacs. In some ovules no female gametophyte was formed. Moreover in Bolesław population 57% of analysed silicules contained two aborted seeds, whereas only in 19% of silicules two mature seeds were observed. In comparison to control material statistical differences were found.

Abnormalities and degenerative processes observed in anthers from Bolesław population were significantly higher than in control material. They included necrosis of whole anthers (20%), disturbances in microsoprogenesis, degeneration of pollen grains (16%) and precocious necrosis of tapetum (10%). Pollen viability in plants from Bolesław was reduced to 86% (in control plants 97%).

The study suggest that unfavorable environmental factors may have negative influence on embryological processes in plants growing on the waste heap.

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Effect of magnetic field on sperm motility and on egg fertilization in sea trout (*Salmo trutta*)

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The magnetic field present in the natural environment is not neutral to living organisms, including fish. It influences adults, while embryos and, even, gametes are sensitive to its influence. The influence of constant magnetic field on sperm motility and on fertilization of eggs of the sea trout (*Salmo trutta*) was examined. Study material consisted of sperm and eggs collected from ready-to-breed fish from River Parsęta (north-western Poland). Gametes were collected from 3 males and 3 females. The sperm used to fertilize the eggs were exposed to a magnetic field of field intensity: 0.5 mT, 3 mT and 5 mT for 30 minutes as well as 24 hours, however, the female gametes were not exposed to the field. Eggs fertilized with sperm not exposed to the magnetic field constituted the control setting.

Sperm motility and percentage of fertilized eggs in each of the variants of the experiment were studied. It was found that the percentage of motile sperm in the samples exposed to a magnetic field for 30 minutes, in the respective values of the field intensity, was 8.16% under 0.5 mT, 3.86% under 3 mT and 10.33% under 5 mT respectively, and 6.13% in the control setting. The percentage of eggs fertilized by sperm kept under magnetic field for 30 minutes was: 59.41% (0.5 mT), 62.38% (3 mT) and 62.31% (5 mT), and 47.65% in the control setting. Where the sperm used to fertilize eggs were kept for 24 under magnetic field of above intensities, the percentage of motile sperm was 7.9% (0.5 mT), 1.66% (3 mT) and 5.83% (5 mT), and 0.6% of motile sperm registered in the control setting. However, the percentage of fertilized eggs for the same variant of the experience was: 22.04% (0.5 mT), 11.07% (3 mT) and 19.33% (5 mT) respectively, and only 0.82% of fertilized eggs registered in the control. A significant correlation between the value of magnetic field intensity under which sperm were kept before used to fertilize the eggs and the percentage of motile sperm, and rise in the percentage of fertilized eggs was observed. Studies carried out helped postulate that exposure of sea trout sperm to a constant magnetic field increases the percentage of motile sperm and their ability to fertilize eggs. Therefore, storing of sperm under magnetic field may be a way for increasing percentage of fertilized eggs, especially in the case of longer intervals between collection of the sperm and egg fertilization.

Oogenesis in viviparous earwig *Arixenia esau* (Dermaptera, Arixeniina)

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In the majority of animals, oogenesis begins with a mitotic division of a of a specialized germline cell, the cystoblast. Divisions of the cystoblast lead to formation of the cyst of sister cells (cystocytes). The cystocytes remain connected by stable connections, termed the intercellular bridges. The ovaries of *Arixenia esau* are meroistic-polytrophic and composed of 3 short ovarioles that consist of a terminal filament, germarium and vitellarium. The ovarioles are attached to a strongly dilated lateral oviduct. In investigated earwig, female germ cell cysts are always composed of two cells only: the pro-oocyte and pro-nurse cells. The nuclei of the pro-nurse cells are large, electron-dense and contain several active nucleoli, in contrast to electron-transparent oocyte nuclei. We show that in the cytoplasm of both cells of the cyst – apart from ribosomes, mitochondria and elements of RER – numerous small polymorphic crystalline structure are located. It should be noted as well, that both cells contain in their cytoplasm the Balbiani body (Bb) composed of numerous mitochondria. Ultrastructural studies indicate that origin of germ cells cysts in *Arixenia esau* follows the pattern described previously in the derived earwigs (Eudermaptera).

In the vitellarium, one ovarian follicle is located. It is composed of a growing (vitellogenic) oocyte and a accompanying nurse cell. The oocyte membrane is equipped with finger-like microvilli. Its cytoplasm contains numerous round mitochondria and large, lens-shaped annulate lamellae. The oocyte is surrounded by two-layered egg envelope and a single layer of follicular cells. On anterior pole of the oocyte, the egg envelope is characteristically folded and forms peculiar specialization with visible channels inside. The follicular cells are flattened and comprise relatively big nuclei with deeply folded nuclear envelope and several nucleoli inside. It should be noted that more numerous and more prominent invaginations of the follicular cell nuclei are located on the side facing the oocyte. Thick ovariolar sheath composed of muscle fibres and plenty of trachea surrounds the whole ovariole.

ACKNOWLEDGEMENTS: The study was funded by the National Science Center (NCN) grant number 2011/01/D/NZ4/00137.

Detection of antigens in stable actin structures during *Drosophila* spermatid individualization by a pre-embedding immunogold labeling method

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During *Drosophila* spermatid individualization, a cyst of 64 syncytial spermatids is reorganized into individual mature sperms by membrane remodeling and removal of cytoplasmic contents. This process is driven by stable actin structures, called actin cones, which are composed of two structural domains, a front meshwork and a rear region of parallel bundles. These two domains are differentially regulated and have different functions. The bundles are required for cone movement, and the meshwork serves a structural role, acting like a "cow-catcher" to exclude the cytoplasm from the sperm tails. One of the proteins that play a key role during individualization is myosin VI, which localizes to the cones' fronts and its specific localization is required for proper actin cones formation and function. However, the mechanism of action of myosin VI in this process remains still unclear. To understand how actin cones are organized and assembled, ultrastructural studies are important to reveal localization of actin regulators relative to regions with different actin filament organizations. Preservation and visibility of actin structures and simultaneous immunolocalization for actin-associated proteins at the ultrastructural level is very difficult, because conditions required for good visualization of actin filaments often preclude antibody labeling. In this work, two different immunocytochemical methods and some modifications of them were tested for localization of antigens in the actin cones. As a result of these studies, we have developed a novel pre-embedding immunogold-silver labeling technique for high-resolution analysis of protein distribution in stable actin structures (Lenartowska et al., 2012). This method is the best for precise analysis of myosin VI distribution in the actin cones and has potential to reveal important information about relationships between actin-binding proteins, membranes, and different types of actin structures.

ACKNOWLEDGEMENTS:This work was supported by the National Science Centre (NCN) grant N303 816240 (to M.L.) and by the National Institutes of Health Grant R01GM-60494 (to K.G.M.).

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Calreticulin expression during pollen-pistil interactions in *Petunia hybrida*

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Calreticulin (CRT) is a highly conserved Ca^{2+}-binding/buffering protein containing a retention signal for ER lumen localization. Although CRT has been suggested to work as a multifunctional protein in many cellular processes in animals and plants, research on CRT has mainly focused on its role in regulating Ca^{2+} homeostasis, signaling, and chaperone activity involved in the secretory pathway. During sexual reproduction of angiosperm, pollen tubes elongate from the stigma to the ovary to deliver non-motile male gametes for fertilization. Ca^{2+} play a fundamental role in pollen tubes growth and guidance within the pistil, but the mechanism of regulation Ca^{2+} homeostasis during pollen-pistil interactions is still unknown. Consistent with its role as a Ca^{2+}-binding/buffering protein, CRT has been suggested to be involved in reproductive events in plants. In the studies reported here, the expression pattern of CRT was determined in relation to pollination and subsequent stages of pollen germination and tubes growth through the *Petunia* pistil. The expression profiles of CRT were obtained by Northern blot analysis of total RNA with the full-length *Petunia* CRT cDNA and by protein blotting with the anti-maize CRT antiserum. The results showed highest levels of CRT transcripts and the protein (1) in mature unpollinated pistils, when a large mass of exudate was detected on the stigma and (2) during first few hours after pollination, when pollen germinated and pollen tubes penetrated the stigma. In pollinated pistils, high level of CRT expression was also detected in ovaries at the end of progamic phase and/or during fertilization and early embryo development. The elevated levels of CRT gene expression have been often correlated with high levels of exchangeable Ca^{2+} at the aforementioned regions of unpollinated and pollinated pistils. Taken together, we conclude that CRT could regulate development and biological functions of the cells engaged in pollen-pistil interactions by modulating Ca^{2+} homeostasis and secretory events.

ACKNOWLEDGEMENTS: This work was supported by the National Science Centre (NCN) grant N303 023 32/1034 (to M.L.).

Development of the posterior rami of the spinal nerves in human embryos aged 6 weeks

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Spinal nerves are attached to their respective segments of the spinal cord by two roots: a sensory, or dorsal root and a motor, or ventral root. The dorsal roots are formed of axons of pseudounipolar cells of the spinal ganglia which develop from the neural crest. The ventral roots are formed of axons of the motor neurons located in the anterior horns of the spinal cord. During early development the spinal ganglia are arranged symmetrically at the sides of the neural tube and, except in the caudal region, are equal in number to the somites.

The aim of the present study was to trace the formation of the spinal nerves and the dorsal rami of these nerves in early human embryos.

Study was made on 15 staged human embryos at developmental stages 15 to 17 (36 to 41 postovulatory days). Serially sectioned embryos were from collection of Department of Anatomy in Poznań. Sections made in three planes, were stained with various histological methods and impregnated with silver.

In embryos at stage 15 (36 days) the spinal ganglia are located at the lateral surface of the neural tube at the level of the sulcus limitans. Thick spinal nerves divide in the ventral and dorsal rami. The communicating rami are well developed. The dorsal rami of the spinal nerves enter developing deep muscles of the back. They traverse the muscles and give off slender twigs ramifying under the surface. During stages 16 (39 days) and 17 (41 days) large fusiform spinal ganglia migrate to the level of the anterior horns of the spinal cord. The dorsal rami of the spinal nerves are thicker than in previous stage and they divide into medial and lateral branches. The medial branches pass more dorsally than the lateral branches and they traverse muscles passing to the superficial tissues.

The effect of inoculum density, culture vessel capacity and medium volume on efficiency of *Narcissus* L. somatic embryogenesis

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Works on *Narcissus* L. micropropagation in liquid media showed that liquid culture systems are more efficient compared to cultivation on solid media (Chen and Ziv, 2001; Selles et al., 1997; Malik, 2008; Malik and Molenda, 2008). The objective of the study was to investigate the effect of inoculum density, culture vessel capacity and medium volume on biomass growth and somatic embryos formation and development in embryogenic callus cultures of *Narcissus* L. 'Carlton'. The clusters of embryogenic tissue obtained on ovary explants under the influence of Picloram and BA were used for the experiment. The clusters were cultured in 100- or 200-ml Erlenmeyer flasks with 20 or 40 ml of medium containing 25 μM Picloram and 5 μM BA. The inoculum density was 1:20 and 2:20. As a control, embryogenic tissue was placed on medium solidified with agar. Culture vessel capacity affected biomass growth and number of embryos obtained. In 100-ml vessels the better growth of embryogenic tissue and higher number of somatic embryos were observed. In cultures of 1:20 inoculum density embryogenic tissue grew more intensively in comparison to higher density cultures. Lower initial density in 100-ml vessels favoured also development of embryos. In turn in 200-ml vessels an increase in medium volume and inoculum density caused an increase in the production of somatic embryos.

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How to study the cytoskeleton failure in the germline cysts of clitellate annelids?

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We can study the influence of given substances on cell and/or organisms using a lot of methods. Starting from breeding them in controlled, laboratory conditions, through using injections into animal's body to in vitro method to cultivate cells in a specific conditions.

In Clitellata (Annelida) germline cysts have a specific and conserved pattern of organization. As a rule, each cell in a cyst (i.e. cystocyte) has only one cytoplasmic bridge (ring canal) connecting it to the central cytoplasmic mass, the cytophore (Świątek et al., 2009). It seems obvious that the cell cytoskeleton is also responsible for cyst architecture. Thus, the main goal of our researches is to analyze the influence of cytotoxic substances affecting microtubular and microfilament cytoskeleton (colchicine, nocodazole, cytochalasin D) on the architecture of germline cysts in clitellate annelids. For our studies we chose male germline cysts from *Dendrobaena veneta*, an earthworm that is easy to breed in the laboratory conditions. During experiments we tested several methods, to find the best one to provide a cytotoxin without damaging target cells and to fully control the dose and exposure time of given cytotoxine to target cells. In our opinion the most successful method is in vitro cultivating for a short period of time (6–48hrs.) of dissected seminal vesicles, which are filled with developing male cysts. We used special breeding solution (modified Dulbecco's Phosphate Buffered Saline) with the proper cytotoxin concentration. We believe that our method is a good tool to study not only the role of the cytoskeleton in germline cysts organization but also helps us to study the other details of germline clusters architecture.

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Effect of abscisic acid on the tulip somatic embryogenesis induction

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Embryogenic callus of tulip cv. 'Apeldoorn', obtained from ovary explants, was put on a proliferation medium supplemented with abscisic acid (ABA) or fluridone (inhibitor of ABA biosynthesis) for one week. After that time the callus was treated with lower level of growth regulators, in comparison with proliferation medium (5 μM picloram and 1 μM BAP instead of 50 μM picloram and 0,5 μM BAP), in order to produce somatic embryos. Within 3 months of the embryogenic tissue cultivation, the effect of growth regulators concentration (1 or 10 μM ABA, 10 or 100 μM fluridone) and their post-treatment effect on embryo differentiation and tissue proliferation were investigated. The number of torpedo-shaped embryos, obtained from 1g of callus, was also examined.

After one week higher percentage of explants (50%) forming globular embryos were observed under the influence of 10 μM ABA, in comparison with other growth regulators. During the further cultivation there were no significant differences between the post-treatment effect of ABA and fluridone on somatic embryos formation, until the 13th week, when higher percentage of explants (35%) forming globular embryos were observed under the influence of 10 μM fluridone.

During the first week 10 μM ABA significanltly decrease the embryogenic tissue proliferation (GV = 0,1), in comparison with control (GV = 0.25) and both fluridone concentration (GV = 0.24 and 0.27).

10 μM fluridone had a positive impact on number (8,5) of torpedo-shaped embryos after the first month of cultivation.

Characterization and expression of a cDNA encoding a seed-specific metallothionein in winter rape

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Metallothioneins (MT) are small nucleus encoded proteins capable of heavy metal complexing with the cysteine rests and are responsible for metal ions homeostasis in the cells (Cobett and Goldbrough, 2002; Koszucka and Dąbrowska, 2006). On the base of the cysteine residues position (Cys) the plant metallothioneins are divided into four types (MT1 – MT4). Our studies allowed to identify new metallothionein gene (*BnMT4*) in the winter rape (*Brassica napus* L.). The analysis of predicted amino acid sequence showed the presence of numerous Cys in order typical for type 4 MT. The nucleotide sequence of *BnMT4* shows 89 % and 86 % homology with *A. thaliana* and *A. lyrata* subsp. *lyrata* genes respectively. The predicted amino acid sequence of *BnMT4* and *A. thaliana* are in 80 % identical. The sqRT-PCR expression profile show no *BnMT4* transcript in the rape cotyledons, leaves, flowers, roots, hypocotyls, and pods. As the expression *A. thaliana MT4* genes occurs only in the seeds, we have studied the *BnMT4* activity in dry and germinating seeds. The high transcript level was observed in dry seeds and remained up to 6 hrs of imbibition. After the next 6 hrs it decreases by half. The minimal transcript level was reached in the 24 hr of germination and was almost 5 times smaller then in the 12 hr. In the 36 and 48 hr amount of *BnMT4* mRNA increases 2.5-fold but in the five days old seedling stays at the minimal level. The functionality of *MT4* rape gene was studied in *E. coli*. pET21-*BnMT4* construct transformed into bacteria improved their growth ability in the copper medium. The obtained results suggest that *BnMT4* is involved in plant development through Cu ions accumulation in the late germs and the control of the first phases of rape seedling growth.

ACKNOWLEDGEMENTS: This work was supported by the Ministry of Science and Higher Education of Poland, grant: N N310 285337.

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Embryonic development of Marmokrebs (*Procambarus fallax* forma virginalis, Hagen 1870)

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Marmokrebs are very interesting objects of study because of the possibility of parthenogenetic development. Attention was paid to him in the 90s the last century when a German pet shops came as breeding animal. Currently, the wild population of this crayfish exist in Eastern Europe, North America, Madagascar and Japan (Jones et al., 2008; Faulkes, 2010). This species due to the specific way of reproduction, high fecundity, small food selectivity and ease of adaptation to different environmental conditions is treated as a potentially invasive species. It is believed that it could threaten like native populations of aquatic invertebrates and fish (Scholtz et al., 2003).

Research on development of marmokreb were conducted in laboratory conditions in an aquarium with a capacity of 90 liters. In each breeding aquarium was placed on three females. After laid eggs the females were transferred to a separate tank. Water temperature which kept incubation female was about 22°C and pH 7.90–8.15. In order to observe the successive stages of development were taken from the incubating female three eggs every 12 hours. It was made biometric measurements of embryos and photographed the next stages of development.

It was found that the breeding conditions *Procambarus fallax* development take about 30 days. Eggs have spherical shape with diameter of 1.5 mm. Identified 10 embryonic stages, which coincide with the observations of Alweys & Scholtz (2006).

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Further studies on causes for poor seed setting in alfalfa

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One of the limiting factors in alfalfa (*Medicago sativa* L.) breeding is low seed setting. Due to this breeders can not overcome the slow selection response in this species. One of strategies used to face this problem is to explore alfalfa inflorescence mutants. Long peduncle (*lp*), branched raceme (*br*) and top flowering (*tf*) inflorescence mutations increase the flower numbers per inflorescence but they do not augment the seed setting per flower. Recently we have shown that both the poor fertilization effectiveness and gradual ovule degeneration resulted in poor seed set in the mentioned genotypes of *M. sativa* (Mól et al., 2011). Here we focus on the analysis of pollen tube growth in relation to the regular ovule and embryo sac formation. No evident differences in pollen tube germination were found in the inflorescence mutants. The *lp*, *br*, and *tf* mutants showed higher frequency of ovule degeneration when compared to the reference cultivar Radius. Thus, the effect of higher flower number per inflorescence was reduced in the mutants by a higher frequency of ovule degeneration. Disturbed ovule and embryo sac development might cause weak pollen tube attraction by the ovules and lower fertilization efficiency in the genotypes investigated.

Acknowledgements: This work was partially supported by the Poznań University of Life Sciences and Adam Mickiewicz University in Poznań interdisciplinary research project No. 2/02.WI/06.

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Cytoskeleton configurations in the embryological structures in *Taraxacum atricapillum*

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The organization of cytoskeleton in the female reproductive cells has been examined in a few sexually reproducing angiosperm species. Research data on the pattern of organization of cytoskeleton in the cells of the apomictic embryo sacs are scarce. To the best of our knowledge, the configuration of the tubuline cytoskeleton in the cells of asexual embryo sac has been analyzed e.g. in an obligatory apomicts *Chondrilla juncea* (Kościńska-Pająk and Bednara, 2006) and *Taraxacum alatum* (Kościńska-Pająk et al., 2010).

The genus *Taraxacum* Wigg forms a polyploid complex with diploid up to dodecaploid representatives, in which the ploidy level and the mode of reproduction are strongly correlated: sexuality is linked to diploids, whereas the polyploids are usually apomictic. Therefore, *Taraxacum* can be studied as a model genus for comparative analysis of configuration of cytoskeleton in the embryo sacs of sexual and agamosperms species. Here, we present the results of preliminary observations of tubuline cytoskeleton in the cells of female gametophyte and in the male reproductive cells in triploid species *T. atricapillum*. The microtubule configurations were observed in different stages of the megasporogenesis and embryo sac development and also in the microspores. The presence of the microspores with well developed cytoskeleton was surprising, particularly in the light of the fact that *T. atricapillum* is considered as the male-sterile species.

ACKNOWLEDGEMENTS: The research was supported by grant N-N303-807940 of the National Science Centre (NCN).

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Spatial and temporal distribution of homogalacturonans in the ovule of *Hyacinthus orientalis* L. before and after fertilization

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Homogalacturonans (HG) are complex cell wall polysaccharides which play a role in multiple plant development processes. Previous studies indicated changes in HG distribution during the progamic phase in both angiosperm (Lenartowska et al., 2011) and gymnosperm (Rafińska and Bednarska, 2011) reproduction.

The aim of this work was to determine the spatial and temporal distribution of homogalacturonans in the ovule cells of *Hyacinthus orientalis*. The research was carried out using immunofluorescence techniques. Three categories of monoclonal antibodies were used to perform the localization of distinct HG classes: JIM7 – against high-methylestrified HG epitopes, JIM5 – against low-methylestrified HG epitopes and 2F4 that recognizes Ca^{2+}-associated HG epitopes.

Before fertilization in the walls of the somatic cells, both nucellus and integuments, only high-methylestrified HG were localized. An increasing concentration of this category pectin towards the micropylar canal and integument's cells was observed. In the embryo sac cell wall all investigated HG were present. Among the egg apparatus cells walls distinct differences were visible in the localization of the pectins. In the synergids cell walls both high- and low-methylestrified homogalacturonans and Ca^{2+}-associated HG occurred. The strong signal from these pectins was clearly seen in the area of the filiform apparatus. In the egg cell no fluorescence from all investigated antigens were observed. The presence of Ca^{2+}-associated pectins in the filiform apparatus of the synergid correlated with high concentration of these ions which suggest an essential role of homogalacturonans in calcium-dependent interaction between the male and female gametophytes.

After fertilization the distribution of all classes of homogalacturonans in the cell walls of the ovule somatic cells wasn't changed. In the synergids decrease of the high-estrified HG and increase of the level of the low-estrified HG in the area of the filiform apparatus was observed. On the other hand in the embryo sac the gradual increase of the high-methylestrified HG fluorescence in the zygote's cell wall was visible. This indicates the synthesis of new wall around the zygote and importance role of homogalacturonans in polyspermy block.

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Calreticulin expression and localization in female gametophyte cells of *Hyacinthus orientalis* L. before and after fertilization

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It is well known that intracellular Ca^{2+} levels are important for sexual reproduction in angiosperm. Calreticulin (CRT) is a Ca^{2+}-binding/buffering protein involved in pollen-pistil interactions and in pollen tube growth and guidance. Mechanism of regulation Ca^{2+} levels in female gametophyte cells is still unknown.

The aim of the present investigations was to determine in vivo the expression and distribution of CRT in cells of the *H. orientalis* embryo sac before and after fertilization. CRT mRNA was localized by fluorescent in situ hybridization and CRT protein by immunocytochemistry techniques.

The obtained results have shown that CRT expression is present in all cells of the embryo sac during successive stages of its functioning. The highest level of the transcripts was observed in cells before anthesis and it decreased during period of flower receptivity. In the progamic phase the different levels of mRNAs were present in the synergid's cytoplasm. Usually in one of them the level of the labelling was higher. In the egg cell and central cell – targeted by sperm cells the level of transcripts was low and localized in the cytoplasm. Fertilization induced growth the level of CRT mRNA in the zygote and the forming endosperm. In divided zygote the high level of transcripts was observed in the cytoplasm and in two-nucleate endosperm in the nucleoplasm and the cytoplasm. In degenerated synergids the low levels of CRT mRNA were observed.

During the period from anthesis to the progamic phase the high level of CRT protein was localized in the filiform apparatus of the synergids. The signal of the fluorescence in the cytoplasm was lower. In the egg cell and central cell the labelling of the cytoplasm was similar. Ultrastructural analysis revealed the presence CRT in ER and dictiosomes. After fertilization the labelling of the filiform apparatus of degenerated synergids was still observed but the signal of the fluorescence in the cytoplasm was decreased. The level of CRT protein in the zygote and endosperm seems to be slightly higher than before fertilization.

In summary, our observations in *H. orientalis* egg apparatus indicates the participation of CRT in the signaling in pollen tube-embryo sac interactions and regulation of the Ca^{2+} homeostasis in cells participating in double fertilization.

Response of *Capsicum annuum* L. DH lines on the in vitro anther culture conditions

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Androgenesis is the process by which haploid plants are obtained from the male gametophyte. The effectiveness of anther culture within the *Capsicum* genus depends on many factors, including genotype, donor plant pretreatment, stage of microspore development and culture conditions. The aim of the study was to investigate the effect of 1 mg/L 2,4-D acid application on donor plants on the effectiveness of androgenesis. Spraying of the plants was conducted in the evening the day before the culture initiation. The experimental material consisted of four DH line of *C. annuum* L. which were derived from interspecific hybrids – the AC5 line from (ATZ1 × CDT)F1, the AT6 line from (ATZ1 × TG)F1, the AP32 and AP40 lines from (ATZ1 × PO)F1. The experiment was carried out according to Dumas de Vaulx et al. (1981) with modifications. All tested genotypes gave positive androgenic response, however depending on the anther culture conditions used. The effectiveness of this process increased from 0.6% (control) to 1.3% after 2,4-D acid spraying for the AP32 line. No difference was observed between control (2.3%) and 2,4-D acid pretreatment (2.7%) for the AP40 line. In contrast, auxin application for the AT6 line decreased the androgenesis efficiency (0.3%) compared to control (1%). Despite of the highest effectiveness of androgenesis was observed for the AC5 line in the control conditions, 2,4-D acid pretreatment did not revealed any androgenesis response. Flow cytometric ploidy level estimation of regenerants showed that among the all tested genotypes haploid and diploid forms were observed.

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Effect of donor plant treatment with 2,4-dichloricphenoxyaceticacid on the anther culture of *Capsicum* spp.

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The anthers of three lines, denoted 335, 342, and 345, obtained through selection among interspecific *Capsicum frutescens* L. and *C. annuum* L. hybrids, characterized by soft-flesh pericarp and capsaicinoids content, were the research material. Twenty individuals of each genotype were treated with 0.1mg·dcm^{-3} 2,4-dichloricphenoxyaceticacid water solution by whole plant spraying one day before bud collection. The ploidy of the plants derived from anther culture as well as callus were determined by flow cytometry as the nuclear DNA content. Used here pungent, soft-flesh, and genetically stable forms were different with regard to the morphological and physiological features. The effectiveness of anther culture of the studied genotypes was low and the main reason for the observed situation was the genetic properties of plant material. In the work by Koleva-Gudeva et al. (2007), which presents the results of the investigation on the induction of embryogenesis on medium CP, the same one that was used in the present experiment, range from zero for hot cultivars to 55% for sweet genotypes of androgenic effectiveness, measured as a relation between embryo and anther number, was observed. For each of the genotypes studied here, higher response of explants from the treated donor plants was observed, which suggests an advantageous impact of 2,4-D. This applies to both callus tissue and embryos. As an effect of this method of anther in vivo pre-treatment, the doubling of embryo number appeared in in vitro anther culture. The conversion of embryo to plant is the crucial point of embryogenesis. High level of the mentioned phenomenon in the present research is promising but limited by the genotype number. The presence of nuclei with 1C and 2C DNA content made it possible to assess the ploidy of plantlets. Proportion of diploids was surprisingly high and caused trouble in the classification of the embryogenesis type. Somatic origin of diploid plantlets cannot be excluded but, on the other hand, spontaneous diploidization during gametogenic embryo formation is also possible.

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The effect of donor plants treatment of 2,4-D acid on induced androgenesis within the *Capsicum* genus

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The effectiveness of obtaining androgenic pepper haploids is the result of many factors, such as: the donor plant genotype, the composition of the media and the conditions of in vitro culture. The aim of this study was to investigate result of spraying of donor plants of 2,4-D acid (concentration 1mg/L) on the efficiency of androgenesis induction in *Capsicum* anther culture. Research material were the DH lines derived from interspecific hybrids of (*C. frutescens* × *C. annuum*) F_1 and (*C. frutescens* × *C. chinense*) F_1. Anther culture was conducted on modified Dumas de Vaulx et al. (1981) method. The CP induction medium was supplemented with addition of silver nitrate (5 mg/L). The varied reaction of the genotypes on the culture conditions was estimated. The line derived from the hybrid of (*C. annuum* × *C. frutescens*) F_1 was characterized by the highest natural ability to form haploid embryos (9%). The application of spraying 2,4-D acid reduced the effectiveness of androgenesis to 5.7%. There was also increasing the share of diploid plants among regenerants. In the case of hybrid lines derived from the hybrid *C. frutescens* × *C. chinense* formation of androgenic embryos were found only in the anther culture of line with the red colour of the fruit. Treatment of donor plants of 2,4-D acid increased the effectiveness of androgenesis from 1.3% to 2.3% and the appearance of diploid and miksoploid plants. In the case of lines of yellow and brown fruit, embryogenesis has not been observed both in control conditions as well as after spraying of 2,4-D acid. In the experiment the high efficiency of conversion of embryos in the plant was observed. Most of the embryos (71%) developed properly and 39 plants were obtained.

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Organization of F-actin cytoskeleton during fertilization in *Alisma plantago-aquatica* and *Sagittaria sagittifolia* (Alismataceae)

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Organization of actin cytoskeleton in embryo sac of *Alisma plantago-aquatica* and *Sagittaria sagittifolia* was examined during pollen tube penetration into the embryo sac to determine the potential involvement of actin filaments in fertilization. Material was stained using DAPI to localize DNA and Alexa 488-labelled anti-F-actin to visualize microfilaments. The organization of the actin cytoskeleton during fertilization in both species is very similar. In the central cell F-actin is distributed along transvacuolar strands, also evident in the perinuclear region and at the periphery of the cell. In the egg cell microfilaments are longitudinally aligned in the micropylar and perinuclear areas; in the chalazal cytoplasm F-actin forms irregular mesh which becomes denser at the place of contact with central cell and persistent synergid. At the 2.5 – 3.5 h after pollination, when in both species the pollen tube arrives, the onset of degradation is evident in one of the two synergids what is being manifested by nucleus distortion. In the synergid to which the pollen tube enters actin cytoskeleton undergoes complete degradation which confirms the results of the research carried out on other species. In second synergid which remains intact microfilaments (creating a denser mesh than in the egg cell) aggregate into bundles near the filiform apparatus and in perinuclear area. However, the accumulation of the microfilaments into intercellular spaces at the chalazal end of the degenerated synergid and between the persistent synergid, egg and central cell has not been noticed. This accumulation of the F-actin which appears to trace the pathway taken by the male gametes during their passage to the egg and central cell occurs in a few species (e.g. *Plumbago, Nicotiana, Zea, Torenia*) and is called actin coronas. The universal occurrence of these structures during double fertilization has been suggested. Results of this study call into question this supposition.

Changes in the structure of endothelium of *Lactuca sativa* L. affected by intergeneric crosses or chemical factors

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Endothelium is a somatic tissue present in certain species belonging to 65 families. It demonstrates a high diversity in structure and origin. As shown in the literature, even in some cases somatic embryos may develop from this unique tissue (Kapil and Tiwari, 1978). The aim of this work was to study the endothelium structure in ovules of lettuce after self, cross pollination with *Helianthus annuus* or after chemical treatment with 0.5% 2,4-D. In all combinations endothelial tissues were analyzed under the light and transmission electron microscopy (TEM). Before pollination, embryo sac of lettuce was surrounded by 1-layered endothelium. Changes of the endothelium structure were observed: a) after selfing – when diploid embryos and endosperm were developed, the endothelium formed 2 layers of cells in the micropylar and chalazal region of embryo sac; the presence of endothelium was noticed even in mature seeds; b) after cross pollination with *H. annuus* – embryos and endosperm developed (with a frequency of 15%) and the endothelium was 2-layered. However, when embryos failed to develop, the endothelium contained 7 layers of cells; c) after chemical treatment with 0,5% 2,4-D – endothelium contained also 1–4 layers but only several-celled embryos were apomictically formed (with a frequency of 14%). Caryological analysis of the endothelial cells revealed the presence of haploid (n=9) and diploid (2n=18) cells. Thus our investigations has shown that after induction endothelium tissue reaches a varied ploidy level and has a high proliferation potential.

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Human viscerocranium at the end of embryonic period (stage 23)

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The bones of the viscerocranium develop in the 1st and 2nd pharyngeal arches and are of neural crest origin. They ossify partly endochondrally and partly intramembranously. The maxilla, mandible, zygomatic, lacrimal, nasal, palatine and vomer are almost completely laid down in mesenchyme close to the end of the embryonic period.

The purpose of presented study is to investigate visceral part of the skull at the end of embryonic period proper with special consideration to the process of ossification.

The study was performed on 12 embryos of developmental stage 23. Embryos were from the collection of the Department of Anatomy, Medical University of Poznań. Age of embryos was based on 23 stages and was expressed in postovulatory days. All embryos were embedded in toto in paraffin or paraplast and serial sections of 5 and 10 μm thick were made. Sections were in sagittal, horizontal, and frontal planes. Histological sections were stained with various methods and impregnated with silver.

At stage 23 (56 days) the ossification is well in progress. Intramembranous ossification of temporal squama and frontal bone begins. The most advanced ossification process is in the mandible and maxilla. Ossification of the mandible begins in the body of the mandible close to the mental foramen and progresses to the mandibular ramus. The ossification center of the maxilla appears in its body close to the infraorbital foramen and progresses to the processes of the bone. One ossification center is seen in palatine process of the maxilla. Two ossification centers are also visible in each of the symmetrical incisive bones. One center is located in the palatine process (medial center) and one in the alveolar process (lateral center). Lateral center of the incisive bone and lateral center of the maxilla fuse. Two ossification centers appear in the vomer. Ossification in the lacrimal bone is not observed.

The egg capsule of *Macrobiotus* sp. (Tardigrada, Eutardigrada, Macrobiotidae): morphology and ultrastructure

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The egg capsule of *Macrobiotus* sp. (Tardigrada, Eutardigrada, Macrobiotidae), as in others tardigrades (Poprawa, 2005, 2011) is composed of two shells: the vitelline envelope and the multilayered chorion. The vitelline envelope is thin, electron dense and adheres closely to the oolemma. The chorion consists of three layers: (1) the inner, homogenous of medium electron density; (2) the very thin middle labyrinthine layer; (3) the outer, homogenous of medium electron density with the structure similar to the inner layer. The chorion surface of tardigrades has an abundant sculpture that is important in the taxonomy of Eutardigrada. Regularly arranged conical processes can be observed on the surface of the chorion of the studied species. Their height is from 7.6–7.7 μm and width of the base is from 6.5–6.6 μm. The distance between the bases of the processes varies from 1.65–1.8 μm. The conical processes are empty inside and their tips are flattened. The processes are connected with thick slats forming a network structure on the surface of the basic chorion. There is no network structure on the surface of the conical processes.

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The cell cycle activity during development of Fabaceae seeds

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The proportion of nuclei in G_2 and G_2/G_1 phase of cell cycle are commonly used as a marker of physiological state of seeds (4C/2C ratio), however this ratio does not include endopolyploid nuclei and thus is inaccurate for polysomatic seeds. The aim of this study was to investigate the activity of the cell cycle during seeds development of the selected Fabaceae species, and then determination of molecular markers of seeds maturity that could be used in seed production. Seeds of *Phaseolus vulgaris, Vicia faba* var. *minor, Pisum sativum, Vicia sativa* and *Medicago sativa* were collected 20, 30, 40, 50 days after flowering (DAF) and seeds of *P. vulgaris* and *V. faba* var. *minor* also 60 at DAF because of the longer vegetation period. Embryos were isolated and the proportion of nuclei with different DNA content in the embryo axis and cotyledons were analyzed using flow cytometer. Collected seeds were also dried and germinated according to ISTA rules. During seeds development endopolyploidy level increased, at the point when seeds are physiologically mature. In the all of the investigated species (except *P. vulgaris*) attaining of physiological maturation was connected with the decrease in mean C-value and (Σ>2C)/2C ratio in the cotyledons. The 4C/2C ratio which is commonly used as a marker of physiological state of seeds is not appropriate for seeds expressing polysomaty and therefore the mean C-value and (Σ>2C)/2C ratio is recommended as a molecular marker of physiological state of polysomatic seeds, which can be easy estimated by flow cytometry.

Can autophagy in the midgut epithelium of *Isohypsibius granulifer* granulifer (Eutardigrada: Hypsibiidae) protect the organism against the microsporidian infection?

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Autophagy plays an important role in cell death decisions and can also protect cells by preventing them from degeneration. There are three types of autophagy: macroautophagy (called simple autophagy), microautophagy and chaperone-mediated autophagy.

The midgut epithelium of invertebrates is on the first line of defense against external factors (e.g. toxic substances, starvation, water deficiency, high or low temperature, pathogens). Therefore it plays an important role in the maintenance of homeostasis of the entire organism. One of the responses of midgut cells to stress factors is cell degeneration: apoptosis, necrosis or even autophagy. Pathogens such as bacteria, viruses or microsporidia can enter the animal's body with food and then pass through the midgut epithelium and its basal lamina in order to attack other organs of the body cavity.

During our previous studies on *Isohypsibius granulifer granulifer* Thulin, 1928 (Tardigrada, Eutardigrada) (Rost-Roszkowska et al., 2010) connected with alterations of the midgut epithelium during oogenesis, we found that some of tardigradan specimens were infected with microsporidia. All stages of pathogens occurred in the cytoplasm of the digestive cells in the midgut epithelium: meronts, sporonts, sporoblasts and spores. The process of autophagy was involved in microsporidian elimination, playing the role in the protection of the entire organism (Rost-Roszkowska et al., 2011). Processes of phagophore, autophagosome and autolysosome formation were described with the use of the transmission electron microscope.

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Are there any differences between autophagy in males and females of *Eubranchipus grubii* (Crustacea, Branchiopoda, Anostraca)?

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The cell death plays an important role in the differentiation of both tissues and organs, and in the elimination of unwanted or harmful cells. Many types of cell death have been described: apoptosis, necrosis, necroptosis, entosis, anoikis, mitotic catastrophe, senescence, and autophagy. However mainly apoptosis, necrosis and autophagy occur in the midgut epithelium of Arthropoda (Franzetti et al., 2011). The aim of our study was to analyze autophagy in the midgut epithelium of males and females of *Eubranchipus grubii* (Crustacea, Branchiopoda, Anostraca) at the ultrastructural level with special emphasis on the formation of autophagosomes. We focused our attention on a) the role of autophagy in the proper functioning of the midgut; b) on answering the question of whether autophagy in *E. grubii* midgut plays a role of an agent of cell death or cell survival. Ultrastructural studies were confirmed by the reactions for acid phosphatase (for the light and transmission electron microscopes). Our studies showed (Rost-Roszkowska et al, in press): a) the midgut epithelium of *E. grubii* is composed of digestive cells, while regenerative cells have not been observed; b) Golgi complexes probably take part in phagophore formation; c) fusion of autophagosomes and lysosomes does not appear; d) lysosomes are enclosed with degenerated organelles by membranes of phagophore; e) autolysosomes are discharged into the midgut lumen due to apocrine secretion; f) there are no differences in the process of autophagy between males and females; g) this process protects midgut cells from cell death.

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The stoneflies of the Atlantic Pyrenees. Egg capsule organization and choriogenesis in the euholognathan stonefly *Nemoura obtusa*

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Two groups of Plecoptera (stoneflies): Euholognatha and Systellognatha (Zwick, 2000), inhabiting temperate climate zone (northern hemisphere) (suborder Arctoperlaria) lay their eggs in clean, well oxygenated waters. The insects representing the mentioned groups differ in detail with respect to their environmental needs. The Euholognatha inhabit slowly running waters; in contrast, the Systellognatha prefer rapidly running streams and rivers. The egg capsules (eggshells) of the two groups accordingly differ in their organization. It was established that the systellognathan egg capsules are thick, hard, multilayered and differentiated both radially and regionally (Rościszewska, 2003). The euholognathan ones, are organized much simpler.

Organization of the egg capsule of *Nemoura obtusa* (Euholognatha, studied in this paper) inhabiting Atlantic Pyrenees was compared with the organization of eggshells produced in euholognathan species inhabiting the Bieszczady mountains.

The results of the present paper confirm our preliminary studies (Rościszewska, 1996; Poprawa et al., 2002) and reveal that euholognathan egg capsules are thin, soft, and with poor regional differentiation.

The data obtained on choriogenesis in the ovaries of the stoneflies studied point out to some activity of an oocyte in eggshell secretion. This primary feature supports the opinion that Plecoptera represent the most primitive insects among Neoptera.

Acknowledgements: We would like to thank Ada Jankowska (Department of Developmental Biology and Morphology of Invertebrates) and Danuta Semik (Department of Cell Biology and Imaging) from Institute of Zoology, Jagiellonian University, for skilled technical assistance. We thank dr hab. Stanisław Knutelski for providing the insect specimens.

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The ultrastructural studies of the follicular lumen formation during the thyroid gland differentiation in the sand lizard *Lacerta agilis* L. (Reptilia, Lacertidae) embryos

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The current knowledge regarding the origin and the mechanism of follicular lumen formation during thyroid differentiation in reptiles in comparison with other vertebrate classes is poor (Rupik, 2011). Thyroid follicles, similar to other organs, consist of tubes or spheroid structures of epithelial cells enclosing a central lumen. During the early stages of thyroid gland development, there is no lumen within the presumptive follicles. The key question is how this lumen is formed during thyroid morphogenesis. The literature suggests an "intercellular" or "intracellular" origin of the lumen in thyroid follicles, while the recent studies of developmental biology have presented two predominant mechanisms of lumen formation during the morphogenesis of epithelial organs that are consist of tubes or spheroid structures. One of them is called "hollowing" and the other one is "cavitation". The eggs of the *Lacerta* were incubated in the constant temperature at 30°C and the embryos were isolated, starting at eggs lying and finishing at hatching of the first individuals. The age of embryos was calculated using the table of species development (Peter, 1904). The results of this study showed that the earliest morphogenesis of the presumptive thyroid follicles in sand lizard embryos appears to be the same as that described in embryos of other vertebrate classes; however, some differences were found during the later stages of its differentiation when the follicular lumen was formed. The follicular lumen in sand lizard embryos was differentiated by cavitation just like in grass snake embryos. During thyroid follicle formation in *Lacerta* embryos similarly as in *Natrix* embryos, a population of centrally located cells was cleared through apoptosis to form the lumen. It cannot be excluded that during follicular lumen formation in this snake species, other types of programmed cell death also occurred. This way of follicular lumen differentiation indicates that it has an extracellular origin.

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Reptilian myotomal myogenesis – lesson from the sand lizard *Lacerta agilis* L. (Reptilia, Lacertidae)

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Reptilian myogenesis has been studied in vitro using adult lizard myoblasts and in vivo during the process of tail regeneration. These studies described the morphogenetic process that leads to the formation of the orderly arranged myotomes in the regenerating tail in adult lizards. On the other hand, a literature survey indicated that the pattern of myotomal myogenesis in reptilian embryos is poorly known. The purpose of this study was to establish whether the steps of myogenesis in the sand lizard (*Lacerta agilis* L.) resemble or differ from the corresponding stages of myogenic differentiation in other vertebrate classes. The eggs of the Lacerta were incubated in the constant temperature at 30°C and the embryos were isolated, starting at eggs lying and finishing at hatching of the first individuals. The age of embryos was calculated using the table of species development (Peter, 1904). The results of the structural, ultrastructural and immunocytochemical studies indicateds that: during somitogenesis, the somites were composed of epithelial vesicles with a centrally located somitocoel. At later developmental stages the ventral portion of the somite cortex disaggregated into the sclerotome mesenchyme while the dorsal wall of the somite differentiated into dermatomyotome. At these developmental stages, mononucleated cells of the dermomyotome were Pax3 positive. It was found that the dermomyotome layer formed the dorsomedial and ventromedial lips. The myotome was composed of first mono- and then multinucleated myotubes and small mononucleated cells that occurred in the vicinity of myotubes. These cells exhibited low proliferative potential as revealed immunocytochemically by the use of PCNA antibody. At subsequent stages of myogenesis the mononucleated cells expressed Pax7 protein, the marker of satellite cells and took ultrastructural features characteristic of satellite cells. Some of mononucleated cells contributed to muscle growth being involved in fusion with differentiating muscle fibres. This study revealed similarities of myotomal myogenesis in the representative reptile to the other vertebrates. It strongly supports the theory of myotomal myogenesis conservativeness among all vertebrates.

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Sterility of *Miscanthus giganteus* results from hybrid incompatibility

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Miscanthus giganteus (Poaceae), a hybrid of *Miscanthus sinensis* and *M. sacchariflorus* native to Japan, is an ornamental and highly lignocellulosic bioenergy crop, currently cultivated in the European Union as an alternative source of energy. This grass reproduces exclusively vegetatively by rhizomes or via expensive in vitro micropropagation (Lewandowski et al., 2003). The main objective of the present studies was to find the barriers which prevent sexual seed production based on detailed embryological analyses of the whole generative cycle, including microsporogenesis, pollen viability, megasporogenesis and female gametophyte development.

Sterility of *M. giganteus* resulted from abnormal development of both male and female organs and gametophytes. Disturbed microsporogenesis (laggard chromosomes, univalents, micronuclei) lead to the production of highly non-viable pollen grains. The frequency of viable (stainable) pollen ranged from 55.3% to 13.9%, depending on the test, with lack of in vitro pollen germination. Significant differences in pollen size (25.5–47.6 μm) clearly indicated the great cytological imbalance of the pollen grains which evidently affected pollen germination.

Only 9.7% of ovules developed normally. Degenerations at different stages of megasporogenesis and female gametophyte development accompanied by disturbances in mitotic and meiotic divisions leading to the formation of unorganized or incomplete female gametophytes affected sexual reproduction. In all analyzed ovules neither zygotes nor embryos were found. Enlarged and dividing sporophytic cells of the nucellus located close to meiotic female gametophyte suggested involvement of apomictic processes. With no doubt sexual reproduction of *M. giganteus* is greatly hampered by its allotriploid (2n=3x=57) nature (e.g. Hodkinson et al., 2002). Hybrid sterility, a strong postzygotic barriers prevents seed formation by sexual reproduction in this taxon. Nevertheless, low percent of normal female gametophytes with egg cells and cytologically balanced microspores/uninucleate pollen grains, potentially capable for development, could be sources for in vitro manipulations towards andro- and gynogenesis induction.

ACKNOWLEDGEMENTS: This work was supported by project No 810/NCOST/2010/0 financed by Polish Ministry of Science and Higher Education in the frame of the COST Action FA 0903 'Harnessing Plant Reproduction for Crop Improvement (HAPRECI)'.

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The hepatic cells of millipedes – does the autophagy play an important role in their functioning?

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Invertebrate digestive system, and especially its endodermal part called midgut, plays an important role in detoxification due to its ability to accumulate toxic substances originated from food or the environment (the stress factors) (Rost-Roszkowska et al., 2011). Therefore the midgut is used as the model organ for the analysis of all alterations caused by the stress factors (Malagoli et al., 2010). The midgut epithelium of millipedes is surrounded by a layer of cells called as "liver", "hepatic cells" or "hepatic tissue", which take part in detoxification.

Three species of millipedes (Diplopoda) have been analyzed in order to describe the role of the hepatic cells in the proper functioning of the digestive system: *Julus terrestris, Archispirostreptus gigas* and *Strongylosoma stigmatosum*. The hepatic cells which do not contact each other and do not form any distinct epithelial layer, are covered with glycocalix – the extracellular material produced by hepatic cells. Their cytoplasm in each of analyzed species is rich in glycogen granules, but differences between *A. gigas, S. stigmatosum* and *J. terrestris* are connected with the accumulation of all organelles (mitochondria, cisterns of RER and SER, Golgi complexes, the structure of the cell membrane). Autophagy as the kind of cell death/cell survival occurred. One of the important feature of those cells is their connection with the midgut epithelium: long strands of the cytoplasm protrude the basal lamina and enter the midgut epithelium. The role of such connections, the autophagy and the ultrastructure of the hepatic cells in three analyzed species of Diplopoda would be discussed.

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The structural and ultrastructural studies of the epidermis in sand lizard *Lacerta agilis* L. (Reptilia, Lacertidae) embryos

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The stratifying, multilayered epidermis of all vertebrate species creates the outermost part of the skin that forms a barrier protecting the body from dehydration, mechanical trauma and microbial insults. Lizards, like other squamate species, have the characteristic scaled skin that distinguishes them from the other amniotes. Epidermis of adult squamates consists of six layers, built from different types of cells. The process of skin differentiation in lizards in comparison to snakes is poorly understood (Swadźba et al., 2009; Swadźba and Rupik, 2010; 2012). We have studied the development of integument in sand lizard (*Lacerta agilis* L.). These studies have based on material from our collection of embryos of the grass snake. The eggs of *Lacerta* were incubated in the laboratory in constant temperature at 30°C and the embryos were isolated in regular sequence of time from egg lying till hatching. The age of embryos was calculated using the table of species development (Peter, 1904). Based on structural and ultrastructural investigation, the embryonic development of the sand lizard integument was divided into four phases just like as in grass snake embryos. Similarly as in grass snake embryos all observed changes in the ultrastructure of the cells forming the epidermal layers in sand lizard were associated with the physiological changes that occurred in the embryonic epidermis, such as changing of the manner of nutrition and keratinization leading to the embryonic shedding complex formation.

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Establishment of micropropagation system for *Rumex acetosa* L. and assessment of genetic stability of regenerated plants

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Rumex acetosa L. is valuable and interesting object of scientific research, because it is one of the few dioecious species that have sex chromosomes. Medicinal applications of *R. acetosa* are related to the tannin content, which is useful for skin irritations. Additionally, modern phytotherapeutical preparations contain extracts from common sorrel for treatment of chronic infections and exhibit strong antiviral activity against herpes simplex virus (HSV-1) (Gescher et al., 2011).

The aim of the present work was to develop an efficient method of micropropagation of *R. acetosa* L., cv. Lionski. Fragments (~5 mm) of hypocotyls, roots and cotyledons of 11-day-old seedlings were used as explants. Earlier attempts to induce caulogenesis in *R. acetosa* from explants mentioned above, were not successful (Ćulafić et al., 1987). MS basal medium supplemented with different types of plant growth regulators was used during experiment. The following growth regulators were tested: TDZ, IAA, BAP, KIN and 2,4-D at different concentrations and combinations. Callus induction was noticed on all tested culture media. The regeneration via indirect organogenesis (adventitious shoots formation) was obtained on media supplied with TDZ and IAA+BAP. The highest morphogenetic response was observed on hypocotyls cultured on MS + 0,5 mg/l TDZ (19%). Among all tested explants the highest callo- and morphogenesis was obtained on hypocotyls.

Plantlets regenerated during culture were rooted using 1/2 MS + 0,5 mg/l IBA medium or IBA-solution (100 mg/l). 95% of rooted plantlets were successfully acclimated.

Random Amplification of Polymorphic DNA (RAPD) providing information on genetic diversity of regenerated plants (somaclonal variation) was used. Genomic DNA was extracted using CTAB buffer. Molecular analysis of donor seedlings and regenerated plants was conducted using RAPD markers.

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Development of the skeleton in cypriniforms (Pisces, Cypriniformes)

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Most fish are poorly developed at hatching. During the early larval period they undergo important functional and morphological changes (Koumoundouros et al., 1999; Jafari et al., 2009). Detailed knowledge about developmental osteology of a species is important for the interpretation of the phylogenetic relationships on different taxonomical levels (Blaxter, 1984; Fukuhara, 1992; Löfler et al., 2008).

The aim of the present study was to describe the developmental morphology of the skeleton in the three laboratory-reared cypriniform species: triploid *Cobitis* females (series from hatching till 23 day post hatching), common bitterling *Rhodeus sericeus* (series from 14 stages) and barbell *Barbus barbus* (series from six stages). The collected specimens were cleared in trypsin, differentially stained with alcian blue to reveal the cartilage and with alizarin red to stain bones according to modified method of Dingerkus and Uhler (1977).

In barbell, most of the bony ossifications of the splanchnocranium were present 20 days post hatching. Two separate centres of development were found in the axial skeleton, Weberian apparatus and caudal fin support. Among the median fins, the caudal fin developed first. Its development in loach was closely related to the flexion of the notochord and began with the formation of the hypurals at 3 dph. Dorsal and anal fin development started simulatneously at 4 dph, followed by the fin radials and rays formation. The pectoral fins started to develop as first at 2 dph. The pelvic were the last fins to develop. They appeared at 7 dph. The bony gas bladder capsule started to develop at 12 dph.

The development patterns of the skeleton found in analyzed *Cobitis* females and barbell were similar to that described for other species of Cypriniformes. The common bitterling is a small ostracophilus fish which spawns on the gills of freshwater mussels. Bitterling embryos develop inside the mussel gill chamber for aproximately one month, leaving the mussel as actively smiming larvae (Smith et al. 2004). At this moment they had absorbed the yolk sac, developed pectoral and all median fins and partially ossified bones of the splanchnocranium.

ACKNOWLEDGEMENTS: This work was supported by the project number 0208.806 of the University of Warmia and Mazury in Olsztyn.

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Effect of constant magnetic field on burbot (*Lota lota*) sperm motility

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The aim of the study was to investigate the effect of magnetic field on motility parameters of stored burbot sperm. Sperm was collected from seven burbot males, from lake Dąbie. After collection, the sperm was transported, sperm from each male kept separately, in test tubes placed in isothermal containers with refrigerated cartridges that guaranteed appropriate constant temperature during transport. Sperm motility parameters were tested using a computer assisted sperm motility analysis system – CASA.

The sperm was stored in a temperature-controlled isothermal room. Containers with sperm were kept under constant magnetic field of intensity 5 mT. Analyses were carried out every 24 hours. The following sperm motility parameters were studied: VSL – straight line velocity, VCL – sperm curvilinear velocity, LIN – sperm linear movement.

The average sperm VSL in the control (variant without action of magnetic field) shortly after sperm collection was 45.5 μm/s, but had dropped to 30.8 μm/s after 24 hours. As for variant under magnetic field, VSL after 24 hours was 37.6 μm/s. In the 216th hour of experiment, sperm movement in the control setting had come to complete standstill, while in the case of sperm stored under magnetic field of intensity 5mT, VSL was 37.9 μm/s. Sperm curvilinear motion velocity (VCL) on the first day of the experiment, in the control setting, was 60.8 μm/s, however, had dropped to 47.5 μm/s after 24 hours. VCL in the sample subjected to magnetic field, after 24 hours, was 59.2 μm/s and 49.6 μm/s at the 216th hour. Sperm motility linear movement (LIN) on the first day in the control was 57.2%, but had dropped to 52.6% after 24 hours. For setting under magnetic field, LIN of sperm subjected to a constant magnetic field of intensity 5 mT, after 24 hours, was 54.9% while at the 216th hour was 63.2%, which may indicate that long-term storage of burbot sperm under constant magnetic field help maintain larger percentage of sperm characterized by rectilinear motion.

Obtained results indicate that magnetic field prolongs the period of motility of stored burbot sperm, which may be of significance in reproduction biotechnology of valuable species, in order to sustain their existence under natural conditions.

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Somatic cells in apoikogenic ovariuterus of a scorpion *Euscorpius ithalicus* (Euscorpiidae)

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Female gonad in scorpions exhibits several unusual features. It is formed by a ladder-like network of longitudinal and transverse tubes containing germline cells and their accompanying somatic tissues. Since the female gonad in scorpions is also a site of fertilization and embryonic development it is referred to as *ovariuterus* (Farley, 1998). In *Euscorpius italicus*, a representative of family Euscorpiidae, the ovariuterus is of apoikogenic type. It means that oocytes grow exposed to the body cavity being attached to the ovariuterus by groups of somatic cells forming the stalks (pedicles). Previtellogenic and vitellogenic oocytes are covered by somatic follicular cells that form a single-layered epithelium. In previtellogenesis and early vitellogenesis the follicular cells are flat. At the advanced stages of vitellogenic growth they become cuboidal. Apical parts of follicular cells face the oocyte, whereas their basal parts are supported by the basement membrane of the ovariuterus. At its base the oocyte neighbors directly on somatic cells of the oocyte stalk. The stalks of previtellogenic oocytes are short and broad structures, whereas those of vitellogenic oocytes are much longer and diversified into two distinct parts. One of them, located at the base of the oocyte, consists of elongated cells with prominent big-sized nuclei. The second part of the stalk, called also a transition zone, is thinner and connects with the wall of the ovariuterus. In advanced vitellogenesis the muscles form well developed sheath on the surface of the ovariuterus, composed of two layers: outer longitudinal and inner circular. The lumen of the ovariuterus is lined with the epithelial cells supported by the basement membrane which delimits the epithelial cells of the ovariuterus wall from the externally located muscle fibers.

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Rumex tianschanicus × *Rumex patientia* – one of the most perspective energy plants: micropropagation protocol and flow cytometric analysis of nuclear DNA content

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Rumex tianschanicus × *Rumex patientia*, an energy sorrel is a hybrid of the English spinach (*R. patientia* L.) and Tien Shan sorrel (*R. tianschanicus* A. Los). It is one of the most promising energy crops, used in commercial biomass production.

The aim of the present experiment was to develop an efficient and rapid method of *Rumex tianschanicus* × *Rumex patientia* in vitro regeneration. Fragments of hypocotyls (~5 mm) and cotyledons of 7, 10, and 12-day-old seedlings and fragments of petioles were used as explants. Culture media (MS) supplied with different types of auxin (2,4-D, IAA, dicamba, IBA, NAA, picloram) and/or cytokinins (TDZ, kinetin, BAP, 2iP) were tested. Additionally, media supplemented with different types of sugars (glucose, fructose and sucrose) in different concentration (1–3% and 6%) were used. Histological analysis of cultured explants was made in order to determine meristematic sites location. During experiment, the first micropropagation protocol for *R. tianschanicus* × *R. patientia* was developed. The highest frequency of indirect adventitious shoots formation (82%) was observed on regeneration medium MS+2% sucrose+2,2 mg/l BAP+0,17 mg/l IAA in the case of 12-day-old hypocotyls. Regenerated plantlets were successfully rooted on 1/2 MS+2% sucrose+ 0,5 mg/l IBA and acclimated.

Based on chromosome counting in root-tip metaphases, chromosome number 2n=20 in *R. tianschanicus* and 2n=40–60 in *R. tianschanicus* × *R. patientia* were determined. Nuclear DNA content in *R. patientia* (2n=60), *R. tianschanicus*, *R. tianschanicus* × *R. patientia* and in callus obtained during in vitro culture was measured by flow cytometry. It was shown that the amount of nuclear DNA in hybrid was similar to mother plant (*Rumex patientia*) and that in callus tissue duplication of DNA content occurred.

Efficiency of androgenesis in liquid anther cultures of winter wheat (*Triticum aestivum* L.)

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The efficiency of obtaining androgenic structures in anther cultures of 8 hybrid forms of winter wheat was studied. Liquid medium C17 containing 2 mg/l 2,4-D, 0.5 mg/l KIN and 90 g/l maltose was used, chosen based on the previous experiments, as it proved to be more effective for androgenesis induction than a medium solidified with agarose. The spikes with anthers in microspore stage were put in a solution, containing micro and macro-elements according to N6 medium supplemented with 2.0 mg/l 2,4-D, for 6 days at the temperature of 4°C. In liquid anther cultures the cracking of anthers and the development of microspores was observed using an inverted light microscope. The first microspore divisions were spotted after 3-4 days, few-celled embryoids after a week, and multi-celled embryoids after two weeks. Overall, 6048 anthers was plated, out of which 5955 androgenic structures (average 98.5/100 anthers, depending on the genotype from 29.0 to 199.1) were obtained. Plant regeneration was conducted on the 190-2 medium supplemented with 0.5 mg/l KIN and 0.5 mg/l NAA solidified with agar, applying light for 12 hours a day. In total 710 green plants were obtained, with an average efficiency of 11.9/100 androgenic structures (from 3.9 to 56.1, depending on the genotype), or 11.7/100 anthers (from 4.7 to 21.4). The ploidy level of androgenic plants was measured by determining the amount of nuclear DNA in the cells of leaves, using cytometry. Among the 173 green regenerants 79 were spontaneously doubled haploids (an average of 45.7%, depending on the genotype from 8.7% to 68.2%). All green plants were vernalized and then planted into pots with soil, to assess the effectiveness of obtaining doubled haploid lines.

ACKNOWLEDGEMENTS: This work was supported MRiRW (HOR hn 801-9/11/3).

The fate of nurse cells in ovaries of two species of medicinal leeches (Annelida, Clitellata)

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Within meroistic ovaries the final fate of germ cells united into syncytial cysts is different. One population of cells, usually more numerous, do not continue meiosis, become polyploid and produce a vast amount of macromolecules (mainly RNAs) and cell organelles (as ribosomes and mitochondria), which are transferred via intercellular bridge(s) into oocyte(s) – these are nurse cells. The nurse cells are eliminated by a programmed cell death in the final stages of oogenesis (Baum et al., 2005). Usually one (sometimes more) germ cell in a cyst has a potential to pass meiosis, gather nutrient material and become egg cell, this is oocyte.

In clitellate annelids during early oogenesis the germ line cysts are formed. The cysts have a specific pattern of organization, i.e. each germ cell is connected via only one cytoplasmic bridge to the central anuclear core, the cytophore (Świątek et al., 2009). The morphological studies have shown that two cell categories develop within cysts: nurse cells and oocytes (Świątek, 2008; Urbisz et al., 2010). However, there are no studies devoted to final fate of nurse cells in clitellate ovaries. Using the standard light and electron microscopy techniques and using TUNEL method we found that nurse cells within ovaries of two species of medicinal leeches (*Hirudo medicinalis* and *H. verbana*) are eliminated by apoptosis.

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Ovary organization in *Stylaria lacustris* and *Chaetogaster diaphanus* (Annelida, Clitellata, Naidinae)

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The ovaries of Clitellata are composed of syncytial germline cysts associated with somatic (follicular) cells. The pattern of cyst organization is broadly the same in all studied so far species. The cyst centre is occupied by anuclear, cytoplasmic core, the cytophore. Each germ cell is connected to the cytophore via only one cytoplasmic bridge (Świątek et al., 2009). However, the cyst shape, number of cells within cysts and number of cysts in ovary may differ markedly between clitellate groups (Świątek et al., 2012). Generally, in oligochaetous clitellates (Oligochaeta *sensu stricto*) ovaries are small, conically shaped structures, connected to the intersegmental septum and covered only by a thin layer of follicular cells. This is the so-called "oligochaetous" type of the ovary. To date, only one exception has been described from ovaries of *Enchytraeus albidus* (Enchytraeidae) where, in contrast to "oligochaetous" ovaries, female gonads are composed of numerous ball-like follicles. Each "follicle" consists of one germline cyst, which is enveloped by follicular cells ("*Enchytraeus*" type of ovaries).

Here we present the preliminary results of our studies devoted to ovary organization in Naidinae, a large group of oligochaetous clitellates, which ovaries have never been studied. In two studied species (*Stylaria lacustris* and *Chaetogaster diaphanus*) we found that ovaries have a form of tiny structures composed of a few germline cysts of pre- and meiotic germ cells covered with a thin layer of follicular cells, whereas cysts containing numerous nurse cells and one developing oocyte float freely within the body cavity. Such organization of ovaries differs from these know to date, thus we propose the term "*Stylaria*" for this type of ovary.

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The cytoskeleton of the embryo-suspensor in *Phaseolus coccineus* L.

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The cytoskeleton plays an active role in many cellular processes, like cellular signaling, organelle motility and also subcellular compartmentation during plant growth and development. The aim of the study was to investigate the presence and changes in the cytoskeleton formation during development of embryo-suspensor in *Phaseolus coccineus*. The visualization of cytoskeleton was studied by using indirect immunoassay of microtubules (Mt) and microfilaments (Mf) (Świerczyńska and Bohdanowicz, 2003; Bohdanowicz et al., 2005). The results of immunolabelling were observed under an epifluorescence microscope. The first division of zygote originates two cells. The basal cell (micropylar) forms the massive suspensor (consist of 200 cells), while the other cell develops into embryo-proper. As a result of endoreduplication the level of endopolyploidy increases toward the base of the suspensor and reaches 8192C. The suspensor of this species is convenient model to study cytoskeleton of highly polyploid plant cells. The microtubules and microfilaments were observed from micropylar to chalazal pole of the multicellular suspensor. Actin filaments were formed a delicate network in the cortical cytoplasm of the suspensor cells. Numerous Mt bundles were observed in the cytoplasm of the suspensor cells also. Many of these tubulin filaments were congregate near the cell walls. In the fully differentiated massive suspensor, the microtubules and microfilaments were formed a dense prominent network composed of numerous cross-linked filaments. In the distal region of the suspensor, a distinct tubulin and actin skeleton with numerous filaments were observed. At all stages of suspensor differentiation in embryo-proper cells an abundant cortical network of actin and tubulin cytoskeleton was visualized.

Acknowledgements: This research was supported by grant BW/1412-5-0318-8 from University of Gdańsk.

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Immunofluorescence analysis of histone H3K36 and H3R17 methylation in prophase mouse oocytes

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Development of the germ cells comprises a set of epigenetic modifications of the chromatin, called reprogramming, ensuring that the genomes of the gametes participating in the fertilization possesses the full developmental potential. In the mouse maternal chromatin acquires the ability to support full embryonic development during the growth phase of prophase oocytes.

Histones are subject to various post-translational modifications (also called histone code or nucleosome remodeling) and specific modification states of histone tails correlate with DNA accessibility and transcriptional activity. Therefore, it is likely that reprogramming of the chromatin of the future gametes may comprise histone modifications.

Histone methylation is so far the most complex modification and methylation of the lysine and arginine residues has important consequences for chromatin structure and gene regulation. Lysine residues can be mono-, di- or tri-methylated, whereas arginines can be mono- or di-methylated. In addition arginines can be symmetrically or asymmetrically di-methyleted.

Tri-methylation of histone H3K36 is connected to the process of active transcriptional elongation and it is reported to be enriched in the 3' end of active genes. Methylation of histone H3R17 has been linked to transcriptional activation and H3R17me2 is implicated in the regulation of pluripotent genes during the early mouse development. Both these modifications are strong candidates to contribute to reprogramming.

Through immunofluorescence applying antibodies recognizing histone H3 methylation at K36m3 and R17m2 we have found that both these residues are methylated in the mouse oocytes at the prophase of the first meiotic division. At present we characterize the methylation status of histone H3 at K36 and R17 during the growth phase of the oocytes, i.e. at the phase when chromatin reprogramming is accomplished. The results will be presented.

ACKNOWLEDGEMENTS: This work was supported by research grant No. N N303 301337 from Polish Ministry of Science and Education (MNiSW) to Z.P.

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Histological and morphological description of digestive tract during development in *Neogobius melanostomus*, invasive species in Baltic Sea

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The aim of the project was to describe and compare histological structure of the digestive tract in round goby *Negobius melanostomus*, among three age groups: less than 1 year (LT=41–47 mm), between 1 and 2 year (LT=58–103 mm) and more than 2 years. Moreover detailed description of structure called secretory oesogaster was performed.

In the study 27 individuals were used with total body length (TL) from 41 to 197 mm. Fish were caught during winter 2006 and in the period from June 2007 to February 2008, in Puck Bay (Poland). Fish were anesthetized and subsequently fixed using two methods: 4% buffered formalin and Bouin's solution. The digestive track was dissected, weighted and cut in to sections. Total mass of digestive tracts was ranging from 0.022g (LT= 43.5 mm) to 2.3 g (LT=197 mm). Histological sections were prepared using paraffin method. Series sections (6 μm) were stained by H+E method and AB/PAS. Afterwards samples were photographed and morphometric measurements were taken, on what description was based.

Significant results were obtained. In digestive tract of all age groups in round goby, following sections can be described: oesophagus, secretory oesogaster, anterior intestine, middle intestine, intestinal-rectal sphincter and posterior intestine. Based on detailed microscopic analysis a lack of stomach in round goby was stated. Furthermore, there was no intestinal bulb, structure characteristic for other species of stomachless fish. Obtained results confirmed existence of secretory oesogaster with alveolar glands, what is probably a consequence of loss of functional stomach in round goby. To definitely specify the role of oesogater, other histochemical techniques must be applied, what will be done in further project. As in other stomachless fish, intestine wall consists of mucosa, submucosa, muscle and serosa. Mucosa consists of epithelium and lamina propria. Epithelial lamina is build by loose connective tissue and the muscle layer is build by two muscle lamellae, one circular and one longitudinal. Their arrangement depends on section in which they occure. Stratified squamous epithelium occurs only in esophagus and is replaced in the rest of sections by single cells layer.

Use of clearing-squash technique in embryological studies of *Poa nemoralis*

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The clearing-squash technique was used for an embryological analysis of *Poa nemoralis* collected from natural habitat in Łazy (Little Poland). A rapid clearing technique with the use of methyl salicylate and phase-contrast microscopy, facilitates large-scale observations of the key processes in apomictic species (Young et al., 1979).

The observations of 688 ovules indicate the presence of diplosporous type of apomixis in *Poa nemoralis*.

The main target of the present study was to investigate the changes that occur from the stage of archesporial cell to the differentiation of the embryo sac mother cell.

The transformation of the megasporocyte into the mother cell of the embryo sac with a complete omission of meiosis was connected with an increase in size and elongation of the archesporial cell. A structural reorganization of the nucleus, nucleolus and cytoplasm was also observed.

The mother cell of the embryo sac changed directly into one-nucleate diplosporous embryo sac.

The subsequent three mitotic divisions led to the creation of a mature embryo sac with a three celled egg apparatus, a central cell with two polar nuclei and a different number of antipodals.

ACKNOWLEDGEMENTS: Autor is grateful to Dr. Maria Kościńska-Pająk from the Department of Plant Cytology and Embryology, Jagiellonian University for her very helpful guidance and encouragement.

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Follicular cell diversification in *Euborellia fulviceps* (Dermaptera, Anisolabididae)

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The ovaries of an earwig, *Euborellia fulviceps* are composed of 5 elongated ovarioles of the polytrophic-meroistic type. The individual ovariole has three well defined regions: the terminal filament, germarium and vitellarium. The terminal filament is a stalk of flattened, disc-shaped somatic cells. The germarium is relatively long and comprises individual germ cells, germline cysts in various developmental stages, as well as somatic pre-follicular cells. We found that the cysts located more anteriorly are small and composed of more than two cells, while the "older" cysts are larger are comprise always two cells only (pro-oocyte and pro-nurse cell). This suggests that in *E. fulviceps*, as in other earwigs from families considered basal, the cystoblast divides 3 times resulting in the formation of 8-cell cluster that is secondarily split into smaller 2-cell cysts.

The vitellarium comprises numerous ovarian follicles arranged linearly. The individual ovarian follicles within the vitellarium are separated by prominent interfollicular stalks. Similarly to all other earwigs studied so far, the follicles are composed of two germline cells only: an oocyte and a single, polyploid nurse cell, that are surrounded by a monolayer of somatic follicular cells (FCs, Tworzydło and Biliński, 2008; Tworzydło et al., 2010). During subsequent stages of oogenesis, initially uniform follicular epithelium starts to diversify into morphologically and physiologically distinct subpopulations. In *E. fulviceps* the FC diversification mode is rather simple and leads to the formation of only three different FC subpopulations: (1) cuboidal FCs covering the oocyte, (2) stretched FCs surrounding the nurse cell and (3) FCs actively migrating between oocyte and a nurse cell. We found that FCs from the latter subpopulation send long and thin filopodium-like and microtubule-rich processes penetrating between the oocyte and nurse cell membranes. That suggests that in *E. fulviceps* cells from at least one FCs subpopulation show the ability to change their position within an ovarian follicle by means of active migrations.

ACKNOWLEDGEMENTS: The study was supported by grant: DS/MND/WBiNoZ/IZ/7/2011.

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Structure of the germarial region in the panoistic ovary of the firebrat *Thermobia domestica*

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The ovaries of *Thermobia domestica* are paired and composed of 5 panoistic ovarioles. The individual ovariole has three easily discernible elements: terminal filament, germarium and vitellarium. The germarium is relatively long and comprises germline cells in various developmental stages. The germline cells are separated by long extensions of somatic cells. Notably, we did not find any germ cells connected into germline cysts, as well as intercellular bridges. Histochemical analyses of whole-mounts stained with rhodamine-conjugated phalloidin showed additionally, that apart from significant amounts of F-actin in cortical regions and the cytoplasm, there are no intercellular bridges within the germarium.

The most apical part of the ovariole is filled with relatively small germline cells with rather small nuclei surrounded with a narrow rim of the cytoplasm. The cytoplasm comprises mitochondria, ribosomes and well developed elements of RER. All the organelles within a cytoplasm are distributed approximately uniformly. The "older" cells – located more posteriorly – are significantly larger. Their nuclei are spherical and comprise heterochromatin aggregations and small nucleoli. In the cytoplasm, numerous ribosomes, elements of RER and mitochondria are present. We found that single aggregates of mitochondria and Golgi stalks are located next to the nuclei. Such aggregations are characteristic for young oocytes of many species. They are referred to as the Balbiani bodies (Bbs) after their discoverer or the mitochondrial cloud (Kloc et al., 2004). So far, *Xenopus laevis* is the only example in which the structure and functions of the Bbs have been investigated in details. In this species, the Bb transports germ cells determinants, i.e. the germ plasm and its germinal granules and localized RNAs to the vegetal pole of the oocyte. Therefore, it plays a crucial role in establishment of the oocyte polarity (Kloc et al., 2004). It can be postulated that the prominent Bbs present in female germline cells of *T. domestica* play a similar role in determining the oocyte and future embryo axes.

ACKNOWLEDGEMENTS: The project is supported by the National Science Centre (NCN) grant number IP2011 057171 under the program Iuventus Plus.

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Tissue-specific interactions of the tyrosine hydroxylase first intron with the nuclear matrix proteins

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Tyrosine hydroxylase (TH) [EC1.14.16.2] is a key enzyme in the catecholamine synthesis in the nervous and endocrine systems. It has been shown that correct expression of TH enzyme is required for survival of the animals during the embryonic development and after birth. Its activity determines the proper function of the dopaminergic system engaged in reward-driven learning and controls the course of many physiologic processes. The activity of the TH enzyme and its gene are regulated at nearly all possible levels by transcriptional and post-translational mechanisms in response to changes in the environment. The TH gene expression is modulated by many regulatory factors including cell and tissue-specific ones. We have been postulated that some of them are the nuclear matrix (NM) proteins anchoring the TH gene by its first intron sequence to the nuclear scaffold. To characterize the nature of the NM proteins interacting with the TH gene Southwestern blotting experiments were performed. The NM proteins isolated from the TH active (bovine adrenal medullae) and TH silent tissue (bovine liver) were separated in SDS-PAGE electrophoresis. Labeled DNA fragment covering the distal part of the TH first intron and 151 bp of second exon (-743/-1571) was bound by 63 and 50 kDa NM proteins of both tissues. Five NM polypeptides 112, 83, 54, 45 and 38 kDa binding the same TH fragment were specific for the adrenal medulla. The NM proteins 79, 71, 40 and 32 kDa isolated from the bovine liver had also an affinity to the molecular probe. Our results suggest that the bovine TH gene chromatin is permanently attached to the nuclear matrix structure by the first intron region. Some residents of the anchoring complex are tissue-specific and their presence correlates with the TH transcriptional status. It is possible that some proteins exclusively found in the bovine NM from liver or adrenal medulla may be responsible for silencing or activation the TH gene expression respectively.

Freezing resistance in fertilized eggs of the rainbow trout (*Oncorhynchus mykkis* Walb.)

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Fertilized eggs of rainbow trout were exposed to freezing temperatures for a short period, and content of CL- ions in water was determined using the Mohr technique. We calculated differences in egg weight, and fluid in perivitelline space after freezing and try to answer to the question: what consequences freezing exposure would bring to early embryogenesis?

The eggs exposed to freezing temperatures for a short period of time (20 min.) lose some water, as evidenced by their weights after exposure being lower relative to the initial values. The loss of water was not too pronounced and never exceeded 1% of egg weight. However, considering the fact that perivitelline fluid makes up about 14% of the egg volume, appropriate calculations would show that the perivitelline fluid volume in the eggs exposed to freezing temperatures was reduced down by 1.33–5.43%.

A pronounced increase in the content of Cl^- ions, up to 122.0 mg Cl^-/dm^3, was recorded in the unfrozen water (around the eggs), compared to 20.5 mg Cl^-/dm^3 in the control. The rate of increase depended on the ambient temperature and on the amount of unfrozen water.

Experiment involving prolonged exposure of cleaning eggs to below – zero temperatures (-2°C) showed that not all the eggs were killed as a result. Although the mortality rate was increasing with time of freezing from 9% on day 1 to more than 50% on day 5 of the experiment, the remaining eggs – when transferred to the control conditions – underwent a regular development of the embryos exposed to the low temperature was found to be almost completely blocked, which was not significantly reflected in the time of incubation as measured in °D (from 308 to 352), but affected the subsequent development and organ formation.

Experiments involving observations of the behaviour of the fertilized rainbow trout eggs showed that a brief period of ambient temperature reduction did not preclude survival of the embryos. Factor enhancing survival include:

- Increase in osmolality of water surrounding egg aggregations during freezing
- Loss of water from the perivitelline space
- Thermal shock and heat emission accompanying freezing.

Paternity identification in intergeneric hybrids of *Salix* × *Populus* using simple sequence repeat (SSR) markers

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The objective of this research was to use simple sequence repeat (SSR) markers to study the parental origin of intergeneric hybrid plantlets of *Salix viminalis* × *Populus tremula*. The hybrids were obtained by in vitro controlled pollination and embryo rescue method (Zenkteler et.al., 2005). Fifteen SSR loci were tested to distinguish parental origin of hybrid progeny. The selected loci were developed from *Populus nigra* (van der Schoot et. al., 2000; Smulders et. al., 2001) and amplified successfully in a wide range of other *Populus* species. We tested the selected loci in different Populus species represented by 3 – 10 individuals and maternal *S. viminalis* donor plants. Six polymorphic loci successfully amplified in *P. tremula* individuals were applied to test paternity of plantlets produced by *P. tremula*. Three of the paternal loci were heterozygotus. The WPMS 16 locus was successfully amplified in both parental plants and progeny. The hybrid nature of plantlets of *S. viminalis* × *P. tremula* was detected by genotypes at loci WPMS 10, WPMS 14, WPMS 15, WPMS 16, WPMS 18, and WPMS 20. All paternal polymorphic loci were contributed to hybrid plantlets. The offspring inherited one allele from the paternal and maternal plants.

Our results show that the six identified loci could be used as diagnostic molecular markers for the presence of paternal genetic material in progeny of crosses between basket willows and aspen. In crosses of *S. vimnalis* and *P. tremula* SSR markers can be a useful tool for identification and selection of hybrid plantlets.

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Immunocytochemical localization of H4 histone acetylated at Lys12 during *Chara vulgaris* spermiogenesis

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Spermiogenesis in alga *Chara vulgaris* is a complex process when spermatids transform into highly specialized mature spermatozoids. During spermiogenesis remodeling of chromatin, conditioned by the exchange of histone proteins into protamine-type proteins occurs. The exchange of nuclear protein takes place during *C. vulgaris* spermiogenesis at stages V-VIII.

Different specific posttranslational histone modifications may have a direct impact on chromatin structure by changing interaction between nucleosomes. Histone acetylation, resulting in chromatine loosening, is required for the formation of DNA strand breaks (Laberge and Boissonneault, 2005). In elongating mouse and human spermatids double DNA breaks appear which coexisted with H4 histone hyperacetylation during change chromatin structure. Remodeling of spermatid chromatin must be preceded by transient DNA breaks since a significant number of nucleosomal DNA supercoils is eliminated from mature spermatids. Positive immunosignals of phosphorylated H2AX histone at Ser139, being the indirect evidence for double-stranded DNA breaks, were shown at stages V–VII of *C. vulgaris* spermatids (Wojtczak et al., 2008).

Immunocytochemical studies of *C. vulgaris* spermatids with the use of antibodies against H4 histone acetylated at Lys12 revealed positive reactions in spermatid nucleus at stages I–VIII. At early stages (I–IV) strong immunosignals in the form of little spots were observed on the whole surface of a nucleus. At the mid stages (V–VIII) these signals were bigger than earlier but their number was smaller. At stages IX, X no positive immunoreactions were observed, similarly as in mouse spermatids (Laberge and Boissonneault, 2005).

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Morphological and histological studies of the hamster tongue during the early postnatal period

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The aim of this study was to investigate changes in the morphology and histology of the tongue during the first 14 days of postnatal life. The study was conducted using light microscopy and scanning electron microscopy. We analyzed the types, distribution, shape and size of lingual papillae and the degree of epithelial keratinization on the dorsal surface of the tongue of newborn, 3-day, 7-day and 14-day-old hamsters.

It was found that the thickness of the horned layer of the epithelium undergoes the essential growth (by 255%) during the first 7 days postpartum, and the greatest degree of epithelial keratinization shows the apical part of the tongue.

On the dorsal surface of the apex and body of the tongue numerous filiform papillae are present, and their shape and size change during development. In newborn animals filiform papillae are short with a round top. With age, they become higher and wider, and their apex becomes more pointed. The largest filiform papillae, known as conical, are located on the intermolar eminence. These conical papillae are arranged in a rosette and for the first time it can be seen in the 3-day-old hamsters Among the filiform papillae less numerous fungiform papillae are placed. They are scattered irregularly on the apex and body of the tongue and in the middle part of the papillae a single taste bud is present. In newborn animals fungiform papillae are poorly developed and less numerous than in older ones.

On the anterior part of the root, in the median line of the tongue a single oval vallate papilla are situated. In the epithelium of the lateral part of this papilla taste buds are located. The number and size of taste buds increase with the age of hamsters.

In 7- and 14-day-old specimens, in the posterior-lateral surface of the tongue there are foliate papillae. The scanning electron micrographs demonstrate that these papillae are separated by deep, longitudinal grooves. There are three parallel grooves on each side of the tongue.

Organogenesis induced by hormones in cultured in vitro *Arabidopsis thaliana* genotypes

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Arabidopsis thaliana is the model plant. The aim of this study was to compare the response of wild *Arabidopsis thaliana* genotype Columbia and *At1g67580* mutant (a homozygotic *Arabidopsis* knock-out line, containing a T-DNA insertion in the first exon of *At1g67580*) on in vitro culture conditions. The *Arabidopsis At1g67580* gene encodes a Ser/Thr protein kinase expressing a significant similarity to animal and human cyclin-dependent (PITSLRE) kinases. The influence of the *At1g67580* mutation on developmental processes in *A. thaliana* was previously studied (Kuta et al., 2008). Totally, 200 hypocotyls and 220 cotyledons of 3-day-old seedlings of both genotypes were cultured on MS medium supplemented with 2,4-D and than on TDZ. The initiation time of callus induction, intensity of callus proliferation and formation of trichome-like structures on MS + 2,4-D (2 mg/l) on hypocotyls and cotyledons of 3-day-old seedlings were genotype dependent, similarly as frequency of caulogenesis on MS + 0.5 mg/l TDZ, the vitality of regenerated shoots, their rooting on MS + IBA and 1/2 MS + 2% saccharose media and acclimatization of the regenerants. Six regenerated plants from hypocotyls of wild genotype Columbia were acclimatized but non of mutants. They closed short living cycle forming flowers, fruits and yielded seeds.

The estrone (female mammalian sex hormone) induced direct organogenesis contrary to auxin (2,4-D) influencing callus proliferation.

In conclusion: 1) regeneration by shoots or by somatic embryos is strongly dependent on culture medium composition and explant type. In the present experiments shoots were formed but not somatic embryos which developed on young embryos on Gamborg medium (Gaj, 2001), 2) mutation of *At1g67580* gene evidently influenced mutant explants reaction on culture conditions. On callus forming on both mutant explants neither shoots nor roots were formed 30 days after transfer on regeneration medium with TDZ. In further experiments the duration on regeneration medium should be prolonged as mutant explants could show delayed reaction.

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The periplasm in the enveloped oocytes of sturgeons (Acipenseriformes) – cortical alveoli and cortical granules

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In the previtellogenic oocytes of sturgeons within the endoplasm two distinct zones can be distinguished: the centrally located granular cytoplasm (containing cisternae of endoplasmic reticulum, ribosomes, Golgi complexes and mitochondria) and the more externally located homogeneous cytoplasm (organelle-free) (Zelazowska et al., 2007). During the oocyte growth the granular cytoplasm expands towards the plasma membrane. In a consequence, in the periplasm of enveloped oocytes of sturgeons (the Russian sturgeon, *Acipenser gueldenstaedtii*, and the Siberian sturgeon, *A. baerii*) the remnants of the homogeneous cytoplasm are present. These remnants become surrounded by the cisternae of endoplasmic reticulum and form cortical alveoli. There are also numerous vesicles resembling lamellar and residual bodies as well as the vesicles of yolk in the periplasm.

During formation of egg envelopes, due to the accumulation of lipid droplets and yolk platelets in the endoplasm, all of the alveoli are translocated to the periplasm. Here, they grow and mature. The "matured" cortical alveoli are a membrane-bounded irregular vesicles comprising an electron-lucent, homogeneous and finely granular content. In the periplasm and between the cortical alveoli numerous cortical granules are located. The cortical granules are produced by Golgi complexes and contain acidic mucopolysaccharides. During the sperm entry into the oocyte the cortical granules are responsible for the cortical reaction. The content of the cortical alveoli is released to the perioocytic space (Cherr and Clark, 1985).

The results of a histochemical staining (i.e. silver staining for acidic proteins, periodic acid-Schiff and lectin staining for glycoconjugates) showed that the cortical alveoli contain a proteinaceous material.

Acknowledgements: This work was supported by funds from BW/5/IZ/2010.

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Differentiation of follicular cells in polytrophic ovaries in Sphingidae (Insecta: Lepidoptera) – preliminary studies

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Like in all other butterflies (Lepidoptera) the ovaries in studied hawkmoth species (Sphingidae) are of the meroistic – polytrophic type. Each ovary consists of four elongated ovarian tubes (ovarioles). Three distinct regions can be recognized along the anterior-posterior axis of each ovariole i.e. terminal filament, germarium and vitellarium. Vitellarium, a major part of the ovariole, is a chain of several dozens of egg chambers in progressively advanced stages of oogenesis. Neighboring egg chambers are connected by somatic interfollicular stalks. Each egg chamber is composed of a cluster of eight germ cells interconnected by intercellular bridges and enveloped by a simple epithelium of somatic follicular cells (FCs). In subsequent stages of egg chamber development the FCs become divided into a few distinct subgroups. Main body FCs associate with the lateral oocyte surface. Centripetal FCs can be found at the oocyte/nurse cells interface, while stretched FCs cover the surface of the nurse cells. During previtellogenesis and early vitellogenesis the main body FCs intensively divide. In consequence, their growing number crowds over the oocyte surface to form a pseudostratified epithelium. To accommodate the rapidly growing volume of the oocyte during vitellogenesis main body FCs rearrange to a simple columnar epithelium. Concurrently the most centrally located centripetal FCs form characteristic petal-like rosette assemblage over the anterior pole of the oocyte. These cells contribute to the formation of a micropyle. Although differentiation of FCs in all lepidopterans examined so far seems to follow a similar pathway, behavior of main body FCs in Sphingidae differs from that described e.g. in Pieridae and Nymphalidae. Essential difference consists in a way these cells are organized within the epithelium at the subsequent stages of egg chamber morphogenesis.

Preliminary studies on androgenesis induction ability of *Miscanthus* × *giganteus*

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Miscanthus × *giganteus* is a perennial grass of the Poaceae family that recently, due to its high biomass productivity and low nutritional requirements has become a leading candidate for commercial biofuel/bioenergy production. Important advantage of its introduction is that as natural allotriploid (3n=57) *M.* × *giganteus* is sterile and could be regarded as non-invasive, environmentally safe species. However, due to asexual propagation it displays very little genetic diversity, what makes works on crop improvement difficult. In order to generate new genetic variations, the possibility of doubled haploid production by the process of androgenesis has been studied.

In total, 98 panicles grown in a field or glasshouse conditions were used, from which about 9400 anthers were isolated. Many factors influencing androgenesis induction were tested: (1) the plant growth stage, (2) the panicle pretreatment (low and high temperature stress for 3–21 days) and (3) in vitro culture conditions: type of the induction medium (190–2; C17; KFWC), type and concentration of auxins (2,4-D, Dicamba, Picloram), various temperature and light regimes.

Despite of all efforts, the results were rather disappointing as only single androgenic structures (AS) with the use of only two protocols were received. Moreover, produced ASs had no regeneration ability and degenerated after a few-week culture.

Cytological analysis of isolated microspores indicated that quite high number (30–60%) of microspores was viable (visualization by FDA staining). However, some abnormalities in microspore development were observed (DAPI staining), what suggests that low efficiency of androgenesis induction was the result of typical for the triploids meiosis and mitosis irregularity, during microsporogenesis and gametogenesis, respectively. However, to confirm received results more detailed studies e.g. cytometric determination of microspore ploidy level and microscopic observations of the earlier stages of microspore development are necessary.

ACKNOWLEDGEMENTS: The research was supported by project No 810/N-COST/2010/0 financed by Polish Ministry of Science and Higher Education in the frame of the COST Action FA 0903 'Harnessing Plant Reproduction for Crop Improvement'.

The possible role of ABA in androgenesis induction in triticale (× *Triticosecale* Wittm.)

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Androgenic development is under control of the plant genome but could be significantly modified by many environmental factors influencing physiological state and condition of the maternal plant. One of the most important features determining androgenesis effectiveness is endogenous levels of growth regulators. Abscisic acid (ABA) is one of the plant growth substances, which plays a key role in plant development and adaptation to environmental stresses. Among others, its involvement in microspore reprogramming and switch toward sporophytic development has been suggested.

In the study, the 'Saka 3006'×'Modus' DH mapping population composed of 72 doubled haploid (DH) lines of hexaploid winter triticale (× *Triticosecale* Wittm.) were used to analyse the changes in ABA accumulation in result of androgenic induction treatment. Used DH lines were selected as significantly different in their androgenic responsiveness and stable in their androgenesis induction effectiveness. The analysis was conducted by indirect enzyme-linked immunosorbent assay (ELISA) on anthers isolated from freshly cut tillers and after low temperature treatment (3 weeks at 4°C) that induce androgenic development.

Significant variation ($p \leq 0.001$) in anther ABA concentration was detected in studied DH lines population (0.8–2.2 nmol·g^{-1} DW). In response to low temperature treatment spectacular, above 1.5-times increase in ABA concentration was observed (1.06–5.28 nmol·g^{-1} DW). However, direct linear correlation between the efficiency of androgenesis induction and ABA concentration in anthers was not detected. Moreover, weak but significant, negative correlation between the concentration of ABA in anthers collected from cold-treated tillers and regeneration efficiency was noted. It seems that androgenesis induction requires the specific threshold of ABA level but too high ABA concentration could negatively affect androgenic structure formation and regeneration ability.

Acknowledgements: The research was conducted in the frame of the COST Action FA0604 'Triticeae genomics for the advancement of essential European crops (TritiGen)' and supported by the Ministry of Science and Higher Education (research project 548/N-COST/2009/0).

Diversity of carnivorous plant turion anatomy of the genera Utricularia and Aldrovanda

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Research on the family Lentibulariaceae is experiencing its rapid growth, as follows from numerous molecular and embryological studies. Aquatic carnivorous plants reproduce very effectively by producing turions (winter buds, storage organs) by use of which they overwinter and survive unfavorable conditions. The aim was to show the differences and similarities in turion anatomy in several aquatic carnivorous plants from the genus *Utricularia*: *U. australis, U. bremii, U. intermedia, U. macrorhiza, U. minor, U. ochroleuca, U. stellaris, U. stygia, U. vulgaris*. We compared turion anatomy of these species with turions of another aquatic carnivorous genus *Aldrovanda* (*A. vesiculosa*). We analyzed the following characteristics: presence of bristles, glandular hairs, and storage materials in the cells. The results show that in all analyzed species turion cells were rich in the storage material, especially starch. Among *Utricularia* species, there were only minor differences between the turion structure: e.g. a part of species had many trap primordia, in others they were rare or absent. In *Aldrovanda* turions, trap primordia occurred (one per leaf). Both in *Utricularia* and *Aldrovanda*, turion surface was covered by glands terminated with two cells. In *Utricularia* these glands produced a mucilage, which might help in surviving the winter conditions.

Acknowledgements: This study was funded by grant N N304 002536 from the National Science Centre (NCN). B.J. Płachno gratefully acknowledges the scholarship for Outstanding Young Scientists from the Minister of Science and Higher Education.

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