ORAL PRESENTATIONS

Gustatory organs in the development of direct-developing *Eleutherodactylus coqui* (Lissamphibia: Eleutherodactylidae)

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Knowledge of the morphology of gustatory organs in amphibians concerns mainly indirect-developing (i.e. with a larval stage) Anura and Urodela species (e.g. Nomura et al., 1979; Żuwała and Jakubowski, 1991, 2001). In the ontogeny of these animals the presence of two generations of gustatory organs was revealed. Taste buds occur in the oral mucosa of larvae, whereas a second generation of gustatory organs (taste discs), characterising metamorphosed individuals, is formed *de novo* at the end of metamorphosis (e.g. Żuwała and Jakubowski, 1991, 2001). The aim of the study was to examine whether the number of gustatory organ generations in the development of amphibians is influenced by the type of development.

The direct-developing Puerto Rican Coqui *Eleutherodactylus coqui* was selected for the study. Five embryonic stages, juveniles and adults were used. The study was carried out using routine methods of light (LM) and scanning electron microscopy (SEM).

In the development of *E. coqui* the tongue fold begins to take shape in the lining of the oral floor at about the 11^{th} day of life of the embryo. Epithelial appendages present in other anuran larvae (e.g. Żuwała and Jakubowski, 1991) were not found in this species. Taste discs are formed in the mucosa of the growing tongue and palate. Under SEM the sensory zones of taste discs are visible at the epithelial surface after hatching.

Conclusion: In the ontogeny of the studied direct-developing anuran species, there is only one generation of gustatory organs: taste discs (confirmed by transmission electron microscopy).

ACKNOWLEDGEMENTS: We are grateful to Prof. dr Richard Elinson from the Department of Biological Sciences, Duquesne University for providing us with material for the research.

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Female germ-line cysts in naidines (Clitellata, Naidinae)

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During gametogenesis germ cells are usually interconnected by broad cell junctions – intercellular bridges – and form functional syncytia that are termed cysts or clusters. In females, such cysts may split into individual cells during early oogenesis (e.g., *Xenopus laevis, Mus musculus – panoistic oogenesis*) or germ-cell forming cysts (cystocytes) may be interconnected until late oogenesis. In the latter case, cystocytes usually diversify their ultimate fates – usually only one cell in a cyst becomes an oocyte and the rest become nurse cells (e.g., many insects – meroistic oogenesis). Germ-line cysts have also been described in annelids during oogenesis. Cystocytes form linear cysts (e.g., *Diopatra cuprea*) in some Polychaeta, whereas cysts equipped with a central cytoplasmic mass (cytophore) have been found in Clitellata.

The present study concentrates on representatives of subfamily Naidinae, which belongs to family Naididae (formerly known as Tubificidae). Our studies revealed that germ-line cysts are also formed in two representatives of Naidinae, i.e. *Chaetogaster diaphanus* and *Stylaria lacustris*. The initial stages of cyst development occur within small and paired gonads in which oogonial and early meiotic cysts have been found. However, the cysts detach from the ovary soon and as a result ball-like germ-line cysts can be found freely floating in the coelom. The architecture of the cysts is typical for clitellate annelids, i.e. each cell is connected to the cytophore via one intercellular bridge. The number of cells in a cyst is not constant, e.g., cysts with 17 and 23 cells have been found in *C. diaphanus*. As oogenesis progresses germ cells in a given cyst differentiate into two morphologically different categories: 1) an oocyte that accumulates cytoplasm, yolk bodies, lipid droplets and glycogen granules and 2) all of the remaining cells that become nurse cells.

There is one prominent difference between the naidines that were studied and other Oligochaeta in relation to oogenesis. The germ-line cysts detach very early from ovaries and develop in the coelom in *C. diaphanus* and *S. lacustris*, whereas in other oligochaetous annelids that have been studied to date, only vitellogenic oocytes are able to detach from the ovary and float freely in the body cavity.

ACKNOWLEDGEMENT: This work was supported by research grant from Polish National Science Centre. Contract grant number: DEC-2012/05/B/NZ4/02417.

Differentation of sclerotomes in human embryos aged 5 weeks

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At the begining of the fourth week the paraxial mesoderm is organized into somites. Process of somitogenesis requires so called cyclic genes (Notch and WNT) and fibroblast growth factor 8. The number of somites according to various investigators varies from 38 to 44 and they are never visible at one time. The somites show early polarization and differentiate into sclerotomes, myotomes and dermatomes. Sclerotomes form a loosely woven tissue which gives rise to formation of the vertebral column.

The aim of present study is to trace the differentiation of sclerotomes in staged human embryos during fifth week.

Study was made in human embryos aged 5 weeks (developmental stages 13–15). Embryos were from collection of Department of Anatomy. Age of embryos was established according to Carnegie staging and was expressed in postfertilizational days. Serial sections made in frontal, horizontal and sagittal planes were stained with routine histological methods and impregnated with silver.

In embryos at stage 13 (32 days) the neural tube extends through the whole length of embryo. The notochord ends anteriorly at the level of the hypophyseal pouch and the perinotochordal sheath formed of cells derived from sclerotomes is well developed. The myotomes and dermatomes are present. Within sclerotomes two zones may be distinguished: in the cephalic part appears loose zone of cells and in the caudal part is the dense zone. These zones may be called sclerotomites. Within the loose zone pass the intersegmental vessels and nerves. Between two zones the intrasclerotomic fissures are present. The sclerotomic cells migrate to the perinotochordal sheath. The division of sclerotomes into two zones is not present in the sacral and coccygeal parts which develop in the caudal eminence.

In embryos at stage 14 (33 days) the perinotochordal sheath is divided into two zones which are not opposite the sclerotomic zones. In the occipital region only fourth sclerotome divides into two zones.

In embryos at stage 15 (35 days) in the cervical and upper thoracic parts the zones of sclerotomes form the primordia of the intervertebral discs and vertebral bodies. The upper three occipital sclerotomes fuse and form the primordium of the occipital bone. The notochord is thicker within the future intervertebral discs.

Cytoplasmic RNPs-rich bodies in larch microsporogenesis

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Cytoplasm of eukaryotic cells is a complex and dynamic compartment. Aside from canonical organelles like mitochondria or plastids, it contains numerous non-membrane bound microdomains enriched in RNA and proteins, which contain transcripts at different stages of posttranscriptional processing. These domains are referred as to cytoplasmic bodies and their role is their involvement in posttranscriptional gene expression control in both somatic and germ line cells.

Despite rich knowledge on cytoplasmic bodies in animal cells, their occurence and function in plants is far less understood. Here we present our research results, which revealed the undescribed before intracellular spatial organization of cytoplasmic stage of splicing elements biogenesis.

Our research model were larch microsporocytes (*Larix decidua* Mill.) during first prophase of meiosis. This plant is characterized by extraordinary long duration of meiotic division, which lasts for over 6 months. For in situ investigation, multilabelling techniques of snRNP components like U snRNA, Sm proteins and m3G cap were used, as well as localization of poli(A)RNA and detection of newly formed RNA transcripts by labelling of in vivo incorporated uridine analogue. *In situ* hybridization and immunolocalization methods were used as also TSA signal amplification technique was applied to increase FISH signal.

We have shown for the first time for plants, that the assembly and maturing of U snRNPs (uridine-rich small nuclear ribonucleoproteins) take place in specialized structures referred as to cytoplasmic bodies (CsBs). *In situ* investigation on larch microsporocytes revealed cyclic occurence of cytoplasmic foci enriched in snRNPs components like U1, U2, U4 and U5 snRNA, Sm proteins and m3G capped snRNA. Furthermore, it has been observed, that the presence of snRNP-rich structures in cytoplasm precedes *de novo* formation of Cajal bodies in the nucleus.

Our latest research revealed, that the larch male germ line cells encompass two distinct types of cytoplasmic bodies, which provide different morphology, molecular composition and function. Except from snRNP-rich bodies involved in the maturation steps of spliceosomal machinery elements, we observe bigger, round foci enriched in Sm proteins and poly(A)RNA, but lacking m3G snRNA. We suggest that these structures comprise a new type of cytoplasmic bodies involved in posttranscriptional regulation of mRNA cycle in cytoplasm.

ACKNOWLEDGEMENT: Presented research was supported by grant NN 303799640.

Oogenesis in *Dactylobiotus parthenogeneticus* Bertolani, 1982 (Tardigrada, Eutardigrada, Murrayidae)

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The reproductive system of *Dactylobiotus parthenogeneticus* contains a single ovary and single gonoduct, which opens into cloaca. The sack-like gonad is placed dorsally to the alimentary tract and is attached to the dorsal body wall by two ligaments. The ovary is filled with the developing oocytes, which are accompanied by trophocytes. In *D. parthenogeneticus*, the mixed vitellogenesis occurs. One part of the yolk material is produced inside the oocyte (autosynthesis),while the second part is synthesized in the trophocytes and transported to the oocyte through the cytoplasmic bridges. The histochemical methods have shown that the yolk accumulated in the oocytes contains proteins, polysaccharides and lipids. The process of egg capsule formation begins in the middle vitellogenesis. The eggs of *D. parthenogeneticus*, similar to other tardigrades eggs (Poprawa, 2005, 2011), are covered with two shells: the vitelline envelope and the chorion. The completely developed chorion is composed of three layers: (1) the inner layer of medium electron density; (2) the middle, labyrinthine layer; (3) the outer layer of medium electron density with conical processes. After chorion formation, a vitelline envelope, that is thin and electron dense, is secreted. The vitelline envelope of *D. parthenogeneticus* is of the primary type (secreted by the oocyte), but the chorion is regarded as a mixed type: secreted by both, the oocyte and cells of the gonad wall.

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Balbiani body in oocytes of the pseudoscorpion *Chelifer cancroides* (Linnaeus, 1761) (Chelicerata: Arachnida: Pseudoscorpionida) and its contribution to lipid droplets formation

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The Balbiani body is an accumulation of organelles and molecules formed in a juxtanuclear position that has been found in oocytes of all investigated animal taxa. Numerous comparative studies revealed that although the Balbiani bodies are conservative structures, they exhibit a significant diversity with respect to composition and behavior during consecutive stages of oocyte growth. On a basis on investigations carried out in model organisms (e. g. the fruit fly, *Drosophila melanogaster* and the clawed frog, *Xenopus laevis*), it has been revealed that the Balbiani body is involved in the germ plasm determination. Recently the special attention has been paid to the contribution of the Balbiani bodies in mRNA turnover and storage. However, the definitive functions of the Balbiani bodies await further study.

The aim of our study was to describe the origin, structure and behavior of the Balbiani body in the pseudoscorpion *Chelifer cancroides*. In oocytes of *Ch. cancroides*, the Balbiani body develops during very early stages of oocyte growth (previtellogenesis) in a juxtanuclear position in a form of a cup- like organelle assemblage. Initially, it consists of numerous mitochondria, endoplasmic reticulum, small patches of nuage material (composed of RNP) and multivesicular bodies. With the progress of previtellogenic growth, the Balbiani body enlarges due to the increasing number of constituents. In the vicinity of nuclear envelope the nuage material gathers and forms a prominent aggregate, while smooth endoplasmic reticulum significantly develops and then splits into smaller parts which intermingle with mitochondria. During advanced previtellogenesis the components of the Balbiani body, including the endoplasmic reticulum/mitochondria complexes, disperse within the cytoplasm. Meanwhile, in the ooplasm more and more numerous lipid droplets appear.

Our findings indicate that in *Chelifer* the Balbiani body is a complex and transient organelle assemblage. Since the components of the Balbiani body disperse within the cytoplasm it is tempting to speculate that it serves as a vehicle for transportation of various organelles and molecules to distant sites of the ooplasm. It seems also plausible that complexes of smooth endoplasmic reticulum and mitochondria that originate in the Balbiani body fulfill the role of centers of lipid droplets formation.

Ultrastructural differentiation of the thyroid gland during embryogenesis in sand lizard *Lacerta agilis* L. (Reptilia, Lacertidae) embryos

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The thyroid is structurally conserved in all vertebrate species and is the first endocrine structure to become recognizable during an animal's development. Only a few reports have described the ultrastructure of reptilian thyroid gland during ontogenesis (Rupik, 2011). The eggs of the sand lizard were incubated in the constant temperature at 30°C. The age of embryos was calculated using the table of species development. Throughout 21^{th}–23^{th} developmental stages, the undifferentiated thyroid primordium contained cellular cords, and the plasma membranes of adjacent cells formed junctional complexes. Subsequently at the 25^{th} developmental stage the first follicular lumens started to form. The follicular lumen in sand lizard embryos was differentiated by cavitation. It was of extracellular origin, as in grass snake species (Rupik, 2012). In the middle of embryogenesis (stage 30^{th}) the follicular cells frequently showed apocrine secretion into follicular lumen. The Golgi complex and the rough endoplasmic reticulum (RER) developed gradually and cilia were formed (stages 32^{th} – 37^{th}). In the differentiating thyrocytes of sand lizard embryos similarly as in embryos of grass snake one cilium per cell was found (Rupik, 2013). These cilia lacked central fibres and therefore they had a 9 + 0 formula that suggested that they were immotile.

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 (DOPog-4201-02-94/05/aj). The sand lizard *Lacerta agilis* L. is not included in Washington Convention of 1973, ratified by Poland in 1991(Dz.U. nr 27 poz.112).

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The process of midgut epithelium regeneration in millipedes (Myriapoda, Diplopoda)

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Regenerative cells, which play the role of midgut stem cells, occur in the midgut epithelium of many arthropods. They are responsible for the renewal of the epithelium as a response to stress factors and during metamorphosis and development (Hakim et al., 2010). Regenerative cells have also been observed within the midgut epithelium of myriapods (Camargo-Mathias et al., 2004), but the processes of their proliferation and differentiation have not yet been described.

As the material for the study, we used the species that are easy to collect and/or easy to rear under laboratory conditions: *Julus terrestris* and *Julus scandinavus* (Julidae, Julida), *Polyxenus lagurus* (Polyxenidae, Polyxenida), *Archispirostreptus gigas* and *Telodeinopus aoutii* (Spirostreptidae, Spirostrepsida), and *Glomeris pustulata* (Glomeridae, Glomerida). In the midgut epithelium of *P. lagurus, J. terrestris, J. scandinavus, A. gigas* and *T. aoutii* the regenerative cells are distributed along the entire length of the midgut, between basal regions of the digestive cells. However, in *G. pustulata* regenerative cells form regenerative crypts, which protrude the body cavity. Up to now, the regenerative crypts have not been described in the midgut epithelium of millipedes. The cytoplasm of regenerative cells in all above mentioned species is poor in organelles. Mitochondria, ribosomes, cisterns of the rough or smooth endoplasmic reticulum, vesicles with electron-lucent content accumulate around the nucleus. Regenerative cells have been described using the transmission electron microscopy however, immunostaining methods were used to detect the mitotic divisions.

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What do we know about calreticulin and its role during key events in sexual reproduction of flowering plants?

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In double fertilization, a reproductive system unique to flowering plants, two immotile sperm cells are delivered to the female gametophyte (the embryo sac) by a pollen tube. The male gametes are released into the embryo sac, usually within one of the two synergid cells commonly known as receptive synergid. One sperm cell fuses with the egg cell to generate a zygote/embryo, whereas the other fuses with the central cell to form the nutritive endosperm. Calcium (Ca^{2+}) plays essential signaling, physiological, and regulatory roles during this multi-step process, which comprises three successive phases: pollination, progamic phase, and gamete fusion. Although the sites and mechanisms of Ca^{2+} mobile storage during pollen-pistil interactions have not been fully defined, Ca^{2+}-binding/buffering proteins likely participate to control the local concentration of Ca^{2+}. A good candidate for this function is calreticulin (CRT), a highly conserved lectin-like molecular chaperone that participates in protein folding and quality control in the endoplasmic reticulum (ER) and regulates Ca^{2+} homeostasis in eukaryotic cells. Because CRT is able to bind and sequester Ca^{2+}, it can serve as a mobile intracellular store of easily releasable Ca^{2+} and control its local concentration within the cytoplasm. Indeed, CRT has long been suggested to play a role in plant sexual reproduction. To begin to address this possibility, we cloned and characterized the full-length cDNA of a new *CRT* gene (*PhCRT*) from *Petunia*. The deduced amino-acid sequence of *Ph*CRT shares homology with other known plant CRTs, and phylogenetic analysis indicates that the *PhCRT* cDNA clone belongs to the CRT1/CRT2 subclass. Our studies demonstrate that enhanced expression of *PhCRT* results in the accumulation CRT protein in response to anthesis, pollination, and the late progamic phase and fertilization, when the level of exchangeable Ca^{2+} changes dynamically. From these results, we suggest that CRT plays a critical role in modulating Ca^{2+} homeostasis within the cells that interact during the multi-step process of generative reproduction in angiosperms.

ACKNOWLEDGEMENT: This project was supported by the Ministry of Science and Higher Education in Poland, grant No N303 023 32/1034 (to ML) and founds provided by Nicolaus Copernicus University for the research program of the Laboratory of Developmental Biology.

Male germ-line cysts in earthworms: a good model for modern methods for the visualization of cell components

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During animal gametogenesis, the germ cells are usually organized in syncytial cell cysts (clusters, clones). The germ cells in such cysts are interconnected via specific cellular channels (intercellular bridges), which ensures cytoplasmic continuity between interconnected cells. In clitellate annelids. germ-line cysts are organized in a specific pattern, i.e. the germ cells encircle the central and anuclear cytoplasmic mass (cytophore). As a rule, each germ cell is connected to the cytophore via only one intercellular bridge.

The aim of our study was to examine the organization of the cytoskeleton (F-actin and microtubules) and the distribution of cell organelles (mitochondria, ER) during cyst development. Male germ-line cysts developing in testes and testisacs of an earthworm *Dendrobaena veneta* (Annelida, Clitellata) were the material for the study. In addition to classical light and electron microscopy methods, we used several additional techniques to achieve our goals: 1) life cell staining, i.e. specific markers for ER – ERTracker, mitochondria – MitoTracker, mitochondria and ER – DiOC6 and a TubulinTracker for microtubules; 2) Steedman wax sections were used to analyze the microtubular cytoskeleton; 3) Serial Block Face Scanning Electron Microscopy was used to obtain 3D reconstructions of young (spermatogonial) cysts.

Additionally, an Andromeda confocal spinning disk (by TILL Photonics) was used for the visualization of some of the results of the florescence labeling.

Our studies showed that: 1) The organization of microtubules and organelle distribution (mitochondria, ER) change during cyst development. 2) The F-actin cytoskeleton can be observed mostly in the intercellular bridges and cortical layer of the cell. The organization of the F-actin cytoskeleton does not change during cyst development. 3) Microtubules seem to play the main role in organelle transport.

ACKNOWLEDGEMENT: This work was supported by research grant from Polish National Science Centre. Contract grant number: UMO-2012/07/N/NZ4/01979. We also want to thank prof. dr hab. Grzegorz Wilczyński and his collaborators from Nencki Institute of Experimental Biology in Warsaw for their kind assistance and their invitation to test the Serial Block Face Scanning Electron Microscopy. Additional thanks Kristian Wadel from Till Photonics and Andrzej Makarewicz from Labsoft for making possible to use a Andromeda confocal spinning disk system.

Interfollicular stalk – two ways to make this structure in Lepidoptera

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Paired ovaries of Lepidoptera are of meroistic-polytrophic type and are composed of 4 to 5 ovarian tubes (ovarioles). Each ovariole contains long germarium (the tip of the germarium contains a single stack of postmitotic somatic cells called terminal filament cells) and vitellarium composed of several dozen sequentially more mature egg chambers connected by somatic stalk cells. Initially, during previtellogenic growth of the oocytes, only a few somatic stalk cells separate the neighboring egg chambers. Successively, in vitellogenesis, prominent differentiated interfollicular stalks can be recognized between ovarian follicles in examined representatives of Lepidoptera. In polytrophic ovarioles of *Drosophila melanogaster* follicular stalk cells develop in the very early stages of the egg chamber formation. Egg chamber formation is thus a sequential process that requires coordination between somatic and germline differentiation programs, probably mediated by intercellular signalling between these two cell lineages. In *Drosophila* ovarioles the first differences among the follicle cells are already evident when the egg chamber exits the germarium. Two different somatic cell types can be distinguish: stalk cells that form the interfollicular stalks, the polar cells, located at each pole of the egg chamber. Both the talk and polar cells are determined in the germarium and derive from a single precursor cell type. Moreover, it is known that in *Drosophila* interactions between follicle cells themselves and between follicle cells and the underlying germline cells pattern the follicular epithelium. Correct patterning of the follicle cell monolayer along the anterior-posterior and the dorsal-ventral axis is essential to obtain a functional mature egg and to establish the polarity of the oocyte and, as a consequence, that of the future embryo. Formation of interfollicular stalk in examined lepidopterans starts in previtellogenesis and continues in early vitellogenic stages of the oocyte growth when the oocyte dramatically increases its volume. Follicular cells covering the lateral sides of the oocyte (main body follicular cells) are the source of cells that build the stalks. In examined species of Lepidoptera 2 mechanisms of main body follicular cells rearrangements leading to interfollicular stalk formation are described.

Effective, repeated and quick system of *Cyathea delgadii* propagation by somatic embryogenesis

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Process of somatic embryogenesis occurs in in vitro tissue culture of a great number of seed plants but the regeneration of fern species through somatic embryogenesis has never been reported yet. *Cyathea delgadii* is the first species belonging to Monilophyta clad for which the effective, repeated and quick system of propagation by somatic embryo production was discovered.

Spores of *C. delgadii* were used to start the gametophyte culture. The zygotic embryos and young sporophytes were obtained within a year of the culture. The stipes of 2-frond, zygotic embryo-derived sporophytes were used for induction of primary somatic embryogenesis. Secondary somatic embryogenesis was induced on intact somatic embryos and the stipes of 2-frond, somatic embryo-derived sporophytes. The initial plant material was maintained in darkness. Our results showed that the first-leaf-derived stipe explants which were just 1.5 to 2.5 mm long, and optimal culture conditions including the agar culture on half strength basal MS salts supplemented with 1% sucrose, are the most effective for induction of somatic embryogenesis. It is worth emphasizing that the explants of *C. delgadii* can produce somatic embryos directly on medium without any growth regulators. After 2 months of culture, somatic embryogenesis was induced on about 70–80% of explants with efficiency reaching 13–19 somatic embryos per explant. The somatic embryo production was risen within 10 months of culture without subculture up to 84 embryos per explants due to spontaneous secondary (direct and indirect) embryogenesis. Light microscopic analysis supported by clearing method showed that the somatic embryos derived from single epidermal cells of stipes of young etiolated sporophytes. Our studies suggest that direct and indirect somatic embryo formation from stipe explants on regulator-free medium is most probably related to the endogenous hormones accumulation.

ACKNOWLEDGEMENT: This work was supported by the Polish National Center for Science (NCN), No. 2011/03/B/NZ9/02472

Pre- and post-pollination mechanisms affecting seed formation in alfalfa (*Medicago sativa* L.)

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Slow selection response as a consequence of the poor seed setting in *Medicago sativa* L. is a serious obstacle in alfalfa breeding. Inflorescence mutants with increased flower numbers per inflorescence augment the total seed set but still show low seed maturation per pod. Long peduncle (*lp*), branched raceme (*br*), top flowering (*tf*) inflorescence mutants, and the reference cultivar Radius were used in our studies on callose deposition in developing ovules before anthesis and in the young seeds up to 4 days after pollination (DAP). Another aspect studied here was the early embryogenesis 1–16 DAP.

Callose appeared in the ovules at all flower bud stages as deposits of various size, intensity and positions within the nucellar tissue. No evident differences in pollen tube growth were found between the inflorescence mutants. Callose was observed in the chalazal region of some ovules 2 DAP, and it was often present in the cell walls of hypostase-like structure. Heavy callose depositions expanded over the nucellus in many ovules 4 DAP. However, it was not clear if callose appeared only in the nucellar cells or in both the nucellus and the embryo sac. Fertilized ovules at early stages of embryogenesis 2 DAP were 3–4 times less numerous in the inflorescence mutants than in the cv. Radius. Ovule degeneration progressed in all analyzed plants 2–4 DAP and most ovules were not properly developed both in the reference cv. Radius (62%) and in the lp, br, and tf mutants (69–86%).

In conclusion, the effect of higher flower number per inflorescence on the seed set was reduced in the mutants by a higher frequency of ovule degeneration and lower fertilization efficiency.

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.

Deposition of callose in young ovules during amphimictic and apomictic trace of reproduction

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Callose is a highly impermeable glucan polymer which occurs in various plant tissues as a component of specialized cell walls at certain stages of growth and whose deposition can be also induced locally by physiological stress, wounding, and pathogen infection. Moreover, callose synthesis is an initial symptom of ovule abortion as well as of embryo senescence. It is also known that callose plays a significant role during micro- and megasporogenesis in flowering plants. Callose deposition has also been correlated with the selection of the FM and the main function of callose is to suppress nonfunctional megaspores by isolation.

The deposition of callose in the megaspore mother cell (MMC) wall is a specific feature of angiosperms with the monosporic and bisporic type of female gametophyte development, whereas callose is absent in species with the tetrasporic type of megagametophyte formation. Although in the ovules of the majority of flowering plants callose is a cytological marker of MMC wall and megaspores walls, its role in the megasporogenesis is not fully understood. It has been proposed that this transient isolation of meiotic cells by callose is necessary for a proper meiosis to proceed.

The pattern of callose deposition and degradation during angiosperms megasporogenesis still remains relatively poorly documented. In apomictic species, callose deposition during megasporogenesis was mainly analysed in grasses and it should be emphasized that the patterns of callose accumulation in the ovules of sexual species are not preserved in the ovules of apomicts. The results of these studies suggest a reduction or complete absence of callose in the ovules of apomictic plants in which meiosis is omitted or disturbed. However, within Asteraceae family, callose was detected in the cell walls of megasporocytes and diplodyads in ovules of diplosporous *Chondrilla juncea*. Recently, we documented callose deposition also in *Taraxacum* ovules, both in sexual and apomictic species. It is possible that differences in the pattern of calose deposition within Poaceae and Asteraceae may be related to different apomictic mechanisms which have evolved in those families.

ACKNOWLEDGEMENT: This study was supported by the grant N N303 807940 from Polish Ministry of the Science and Higher Education.

Distribution of AGO1 in mature pollen grains and *in vitro* growing pollen tubes of *Hyacinthus orientalis* L.

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ARGONAUTE (AGO) proteins are integral players in all known small RNA-directed regulatory pathways. In plants, there are several members of ARGONAUTE family, each involved in some specific small RNA biogenesis pathway (such as miRNA, siRNA, piRNA, scanRNA). AGO1 is the one of the 10 known AGO proteins in *Arabidopsis*, which is specifically associated with miRNA biogenesis. Micro RNA (miRNA) are endogenous 21–24 nt RNAs that can down-regulate gene expression by pairing to the messages of protein-coding genes to specify mRNA cleavage or repression of productive translation. They act within the RNA-induced silencing complex (RISC) which contains AGO1.

Recently AGO proteins were found participated in regulation of plant reproduction (e.g. rice, maize, tomato). The aim of the present investigation was to determine the spatial and temporal distribution of AGO1 in mature pollen grain and *in vitro* growing pollen tubes of *Hyacinthus orientalis* L. The detection of AGO1 was performed with immunofluorescence techniques.

Our study has shown the present and changes in distribution of AGO1 in male gametophyte cells. These results indicates that the processes including noncoding small RNAs take place and play important role in post-transcriptional regulation of genes expression in these cells.

ACKOWLEDGEMENTS: The research was supported by the National Science Centre (NCN) grant 2011/03/D/NZ3/00603.

Induction and development of haploid embryos and plants of *Lectuca sativa* after intergeneric crosses and chemical treatment

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Lettuce (*Lactuca sativa* L.) is a very popular and economically valuable leafy vegetable. It is well known that haploids are significant in plant improvement and are important base for the production of homozygous plants. The main aim of this work was to find an effective method of haploidization of *Lactuca sativa* which could be useful for lettuce' breeders. In our investigations we focused on application of some parthenogenetic techniques like – inducing the development of haploid embryos and plants of lettuce by chemical treatment with 8 various inductors and through the wide crossing with 25 species (which mainly representing the Asteraceae family). Pollination and chemical induction of lettuce were conducted in vivo (on receptive stigmas of pistils) and in vitro (on pistils, ovaries, ovules and isolated embryo sacs surrounded by endothelium). Pollen grains of all Asteraceae species, germinated on stigmas of *L. sativa* already 1h after pollination. Induction of development of haploid, globular embryos were stated after pollination with pollen of 20 out of 24 species. It must be stressed that, embryos were developed even if germination of distant pollen did not occur (4 crossing combinations). The highest frequency of embryos was achievied after crossing with *Helianthus tuberosus* L. (16%). Application of seven of eight tested chemical inductors resulted in the development of only several-celled embryos – the most effective was Dicamba (16%). All obtained globular embryos were haploid (n=9) but they did not developed further. The embryo haploidy was verified by: counting of chromosomes in dividing cells and comparison of nucleus perimeter with the diploid ones. The haploid plants of *L. sativa* were regenerated from the callus tissue obtained from the proliferating haploid embryos, cultured *in vitro*. During 4 years of these investigations – 23 haploid plants of lettuce were regenerated. The ploidy level of all obtained in vitro plants were examined by: flow cytometry analysis, KASP analysis of 45 molecular markers, counting chromosomes in dividing cells of roots' apical merystem, comparison of width dimension and density of leaf stomatas and analysis of micro- and megasporogenesis. All the used methods proved to be useful for the confirmation of haploid nature of the regenerated plants.

The number and timing of appearance of the ossification centers in the frontal, maxillary and zygomatic bones

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The maxilla, palatine and zygomatic bones develop from the maxillary process of the first pharyngeal arch. The frontal bone develops within the roof of the developing brain. All these bones ossify intramembranously and form walls of the orbit and most of the viscerocranium. There is no agreement as to the time of appearance and the number of ossification centers in each bone.

The aim of study was to trace the beginning of ossification in each investigated bone during the late embryonic and early fetal periods.

Investigations were made in 25 embryos and 24 fetuses from collection of Department of Anatomy University of Medical Sciences in Poznań. Embryos aged 46 to 56 days (developmental stages 19 to 23) were serially sectioned in frontal, sagittal and horizontal planes. 19 embryos aged between 9 and 14 weeks, after decalcification, were serially sectioned in 3 planes. Five embryos aged 9 to 14 weeks were stained with alizarine red. Serial sections of embryos and fetuses were stained according to several histological methods and impregnated with silver salts.

The first bone to show ossification is the maxilla where process begins at stage 19 (46 days). The premaxilla ossifies from 2 ossification centers: one for the alveolar process and one for the palatine process. The maxilla proper ossifies from 3 ossifications centers: one for the body and alveolar process which appears at stage 19, one for the palatine process which is visible during stage 20 (49 days), and one for the frontal process appearing during stage 21 (51 days).

In the zygomatic bone 3 ossification centers were present. First ossification center for the body appears at stage 22 (53 days). Accessory ossification centers are present in the frontal process and temporal process. Both these centers appear at the end of the 8th and during 9th weeks.

Within the frontal bone first ossification center was present in embryos at stage 20 (49 days). It appeared above the future supraorbital margin of the bone and grows into frontal squama and orbital part.

In fetuses aged 9 to 12 weeks secondary ossification centers in the frontal bone were found: one for the zygomatic process and one for the nasal part.

Comparative anatomy of ovules in Asteraceae

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Special modifications of the ovule tissue and the occurrence nutritive integumental tissue has been described in several Asteraceae species, including the invasive *Taraxacum* species. The main aim of our study was to check whether such modifications might also occur in other genera. According to changes in the integument tissue, we identified three main types of ovules in Asteraceae.

ACKNOWLEDGEMENTS: This study was funded by the grant 2013/09/B/NZ8/03308 from the National Science Centre. BJ Płachno gratefully acknowledges the scholarship for Outstanding Young Scientists from the Minister of Science and Higher Education.

Morphological changes of the ovaries and uterus during the prenatal development in domestic cat (*Felis silvestris catus*)

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The morphological development of the female genital organs in cat is connected with the development of the urinary organs and consists of the following stages: 1) Formation of the mesodermal urogenital ridges, mesonephroses and differentiation of gonads; 2) Formation of the Wolffian and Müllerian ducts; 3) Histogenesis of the ovaries and the wall of the uterus and the uterine tubs. 4) Reorganization of the genital organs in the abdominal cavity, connected with the mesonephroses atrophy, what results in change of the ovaries position and elongation of the uterine horns.

The aim of the study was to reveal the morphological changes during the development of the genital organs in female cat foetuses from 25th to 63rd day p.c., using LM and SEM techniques and also the 3-D reconstruction technique on serial histological sections.

After the 25th day of prenatal development the cat female genital organs start to develop and differentiate. The ovaries on the 30th day p.c. have an elongated shape and arrange ventrally on the symmetrically positioned mesonephroses. The uterine horns pass freely into the uterine tubes and arrange themselves laterally along the mesonephroses. At this developmental stage the ovaries and uterine horns are almost at their entire length combined with the mesonephroses. The free parts of the uterine horns converge in the caudal area of the abdominal cavity and form the body of the uterus. On the 40th day p.c. the mesonephroses are in the phase of the physiological atrophy. The ovaries a slightly increase and contact with the mesonephroses by the developing proper ligament and suspensory ligament of the ovary. The uterine horns elongate and have a V-shaped structure. After the 50th day p.c. the mesonephroses disappear and the mesovarium develop. The asymmetry in the size and position of the genital organs is observed. The left ovary is arranged lower in the abdominal cavity than the right ovary and the left uterine horn is approximately 25% longer than the right uterine horn. The uterine tubs clearly distinguishable from the uterine horns and arrange sinuously at the surface of the ovaries.

Accumulation of poly(A) RNA in the Cajal bodies and nucleus

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The Cajal bodies (CBs) are evolutionarily conserved structures present both in animal and plant cells and involved in the storage and maturation of both snRNPs and snoRNPs, as well as other splicing factors necessary for mRNA and pre-rRNA processing but not directly on the transcription. During the diplotene stage in larch microsporocytes a large amount of poliadenylated RNA (poly(A) RNA) in the nucleus is accumulated. Our previous study showed that in these cells CBs are the site of storage and/or modification of poliadenylated transcripts but are not a place where newly formed transcript appeard. Using double labeling of newly formed transcript and poly(A) RNA we showed that the level of poly(A) RNA in CB varied in different stages of diplotene and is correlated with newly formed transcript level. Appearance of poly(A) RNA containing CBs, followed by period of high transcriptional activity, and precedes the appearance of a large amount of poly(A) RNA in the cytoplasm. We performed single molecule labeling using ELF 97 mRNA *in situ* hybridization method which showed that CBs containing mRNA harboring retained (unprocessed) introns. We also conducted an experiment in which we extended the time of incubation of the larch microsporocytes with BrU. The newly formed transcripts appeared in CB after 3h of incubation. These data suggest that the Cajal bodies are the nuclear domain involved in the regulation of mRNA expression mainly due to translation delay.

The histogenesis of lingual glands in the domestic duck (*Anas platyrhynchos f. domestica*) during embryonic development

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In the domestic duck anterior and posterior lingual glands develop in two different ways. Anterior lingual glands develop by branching the glandular primordia and posterior lingual glands firstly elongate and then start to branch.

The aim of the study is to distinguish stages of the development of lingual glands and their histological characteristic in the domestic duck and also specify the time of the beginning of the secretory activity. The study was proceed on tongues of domestic duck embryos from 9th to 25th day of incubation by using LM and SEM methods.

The development of the lingual glands occurs in 4 stages. The first, epithelial cord stage start on 11th/12th day. Lingual glands primordia are short, solid epithelial cords composed by closely arranged, undifferentiated cells. The second stage start from 14th day in anterior lingual glands and in posterior lingual glands between 15th and 17th day. In this period called dichotomy division stage, the terminal section of glands epithelial cords start to branch and create primary secretory units and the remaining part forms conducting ducts. The third stage – the opening stage of glands epithelial cords starts between 18th and 22nd day. During this stage the embryonic epithelium, firstly transform into glandular epithelium consists of 2 – 3 cell layers and then into simple cylindrical epithelium, like in adult birds. Openings of the lingual glands on the lateral part of the body and lingual prominence and on the root of the tongue start to open about 18th – 21st days of incubation. Secretory activity of the glandular epithelium in anterior and posterior lingual glands occurs on 16th day. Glands, firstly produce neutral glycoproteins, then sulfmucins and sialomucins.

At the end of incubation, the composed anterior and posterior lingual glands of the domestic duck are fully developed and produce mucous secretions, whose composition is similar to adults birds. The distinguished developmental stages of lingual glands in duck differ from pattern described in salivary glands in mammals.

Genetic similarity versus morphological differentiation of well adapted to serpentine soil violets from the Melanium Ging. section (*Viola* L., Violaceae)

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The Melanium Ging. section whose reticulate evolution is based on hybridization and polyploidization comprises 80–100 species, genetically closely related but extremely variable morphologically and with very diverse chromosome numbers. The taxonomy of this group is very complicated and uncertain. A huge number of new intraspecific ranks have been created (species, subspecies, forms) or a number of similar taxa were grouped in collective species (Yockteng et al., 2003). Furthermore, the genetic and morphological diversity of the Melanium violets could be increased by metal polluted environments as estimated in *Viola tricolor* from metal polluted soils (Słomka et al., 2011, 2012).

This study focused on the origin and on the genetic relatedness, morphology and adaptation of several Melanium taxa (*V. aetolica, V. albanica, V. dukadjinica, V. raunsiensis, V. macedonica*) from Albanian serpentine (Ni/Cr) and chalk soils. Phylogenetic analysis based on molecular nrITS markers grouped all investigated species in one clade and indicated their closer genetic similarity to *V. lutea* and *V. arvensis* but not to *V. tricolor* suggested as putative ancestors. This is in accordance with our previous studies on two zinc violets (*V. lutea* ssp. *calaminaria* and *V. lutea* ssp. *westfalica*) (Hildebrandt et al., 2006). Whereas morphological characters evidently separated all species with the exception of *V. aetolica* vs. *V. macedonica*, the phylogenetic relationships among Albanian violets could not be resolved by ITS phylogeny. Neither microstructural flower characters nor pollen heteromorphism proved to be helpful in species identification. High frequency of viable pollen (above 80%) counted in all analyzed species indicated low tolerance costs and high level of species adaptation to metal polluted soils.

ACKNOWLEDGEMENTS: These studies were funded in part by the Dean of the Faculty of Biology and Earth Sciences of the Jagiellonian University (project no. DS/MND/WBiNoZ/IB/5/2013) and by the Polish Ministry of Science and Higher Education (project no. IP2011 050471).

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Applications of X-ray computed tomography in the analysis of the embryogenesis of Arthropoda: the midgut development of *Neocaridina heteropoda* (Crustacea, Malacostraca)

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X-ray Microtomography (XMT) is a non-destructive, computed-aid visualisation technique widely used in many science areas. This method employs electron X-ray conical beam to visualize internal structures of analysed object. During transition through the object, attenuation of X-ray occurs, however, it depends on the material density. The higher density is, the bigger attenuation is observed. The quality of obtained image depends on many variables. Two main issues are critical in this case, size and structure of an object. Limitations of XMT is maximum size of analysed object. The bigger sample is, the lover resolution is able to obtained. Density-differ structure of object is desirable for satisfactory analysis. Homogenous objects, like soft tissues, are difficult to analyse because there are no significant differences in attenuation inside them.

Eggs, larvae and adult specimens of *Neocaridina heteropoda* (Crustacea, Malacostraca) were prepared for the analysis with the use of X-ray microtomography to visualise its internal structure. All developmental stages were scanned with the same procedure. The aim of our study was to analyze the process of midgut epithelium formation during the development of *Neocaridina heteropoda* (Crustacea, Malacostraca). These non-destructive three-dimensional imaging technique allowed us to create the high-resolution morphological and anatomical data in three dimensions. These techniques allow detailed virtual reconstructions of the morphology and anatomy of egg, larva and adult organism and subsequent interactive manipulation (rotation, virtual dissection) and precise analysis of these data.

Sporogenesis and embryo development in apomictic *Poa pratensis* genotypes

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Apomixis in angiosperm plants leads to metromorphous (maternal) offspring and is desirable in agriculture as a way for cloning plants by seeds and stabilization of heterosis (Singh at al., 2011). In this case embryos develop by parthenogenesis from unreduced egg cells in diplosporous or aposporous female gametophyte (embryo sac). Diplosporous embryo sac originates from a generative cell, either directly by mitosis, or indirectly by modified meiosis. Aposporous embryo sac originates from the somatic cell of the ovule. In the both embryo sac types, the differentiation of a normal embryo presupposes a normal differentiation of the endosperm, either on the way of pseudogamy or autonomy.

Diplospory as well as apospory concern only the female reproductive cells and male meiosis can be more or less normal. Unreduced pollen grains are relatively rare and giant ones are occasionally reported.

The objective of this research were the investigations of apomixis in choosen cultivars and strains of *Poa pratensis*. Material used in this research consisted of three varieties namely Alicja, Ani, Nandu and 16 strains i.e. PN/15/R, PN/60/R, PN/162/R, NIB237, NIB252 NIB1703, SKW11, SKW15, SKW16, SKW29, SKW33, SKW35A, SKW35B, 15z/07, 29Z/07 I 32Z/07 of *Poa pratensis*. These materials were investigated by embryological methods to reveal the reproduction pathway in glasshouse and field conditions. Especially, microsporogenesis, macrosporogenesis, embryo development and seed formation were analysed. The data obtained from these observations indicate that both genotype and the environment modified the number of spikelets and the number of kernels per inflorescence. In the greenhouse fertility expresed as the percentage ratio number of kernels/number of spikelets was slightly higher compared to that which was observed in the field. Also the viability of pollen grains was different depending on the genotype and the environment. The data obtained from the observations of sporogenesis, embryo and endosperm development showed that four genotypes i.e. Alicja, Ani, Nandu and PN/60/R seems to be facultative apomicts and the rest twelve genotypes have formed seeds only on the apomictic way.

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Can GFP expression affect cell physiology?

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The green fluorescent protein (GFP) emits stable, species independent fluorescence and does not require any substrates nor cofactors. When fused to another protein, GFP does not lose ability to emit the fluorescence what makes it irreplaceable tag to monitor some complex cellular processes. Therefore GFP is widely used as a live cell reporter in different cell types and organisms including *Drosophila*. It is believed that GFP-transgene expression is innocuous for cells and does not influence endogenous gene expression. Despite this, number of side effects have been reported, suggesting that GFP can affect cell physiology and proper cellular functions.

GFP-tagged variants of myosin VI (MVI) with particular sequences deleted or altered have been extensively used in research on the role of MVI during the last step of *Drosophila* spermatogenesis called spermatid individualization (Isaji et al., 2011). In these experiments, fertile males expressing full-length GFP-MVI transgene (GFP-MVIFL) were used as a positive control for different sterile MVI mutants. To determine whether GFP-MVIFL expression causes any side effects during *Drosophila* spermatogenesis, electron microscopy on cross-sections of testes dissected from the wild-type males (WT) and GFP-MVIFL males (expressing only transgene or transgene in the presence of endogenous MVI) was performed. Comparative analysis showed that GFP-MVIFL expression can induce subtle ultrastructural changes in maturing spermatids when endogenous MVI is absent. Additionally, specific actin structures formed during individualization (called actin cones) were stained with phalloidin and examined by fluorescence microscopy. Measurement of actin cones showed that they were shorter and wider in males expressing only GFP-MVIFL transgene. All these differences seems minor because males were able to complete the individualization process, suggesting there is no serious side effects of GFP-MVIFL expression in maturing spermatids.

Our data confirm that GFP is nontoxic and does not affect proper cellular functions. However, possible side effects of GFP-transgene expression should be kept in mind when using this marker to track cells because the induced fine changes in cellular organization may confound interpretation of functional data.

ACKNOWLEDGEMENT: This work was supported by Polish Ministry of Science and Higher Education grant NN303 816240 (to M.L.).

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Anatomy of pistil and pollen tube transmitting tract in selected species of *Salix*

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Several species of genus *Salix* are important from an agronomic point of view, especially for biorenevable energy cropp. Their biology of reproduction are not yet clear understod.

The aim of the present investigations was to determine the structure of *Salix* pistil in relation to the growth of pollen tube along the transmitting tract, and their fate before/during fertilization. Preliminary studies on style and stigma anatomy of *S. vinimalis*, *S. fragilis, S. cinerea* and *S. rubra* with particular reference to the gynoecial pathway of pollen tube were undertaken using light and fluorescent microscopy. Recent research summarized our data concerning pistil of *Salix* spp. structure. The syncarpous, bicarpellate gynoecium distinctive of the genus *Salix* contains unilocular ovary with parietal placentation, and few anatropous ovules. The short, solid style bears at the top of bilobed dry stigma. A detailed investigation into the enzyme activity revealed that peroxidase and esterase were localised on the dry type stigma surface (Peroxtesmo KO test). In subsequent levels of solid style cross sections has been observed *Salix* gynoecial tissues which scarcely provide nutritional support to pollen tube growth during passage through. The lack of exudate could be regarded as one of causes of pollen tube attrition.