POSTERS

In vitro regeneration abilities of bulb explants of *Lachenalia* 'Romaud' on the initiation culture stage

Anna Bach, Anna Kapczyńska

Department of Ornamental Plants, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425, Poland, e-mail: robach@cyf-kr.edu.pl

The genus *Lachenalia* (lachenalia, Hyacinthaceae) is a relatively new floriculture product – the commercialization of the first improved hybrids, developed in South Africa, occurred in the nineties of last century (Kapczyńska, 2009). The large diversity in flower form and foliage make lachenalia an excellent crop with a potential to be exploited commercially in nearly future. The appearance of such new horticultural product, requires the formulation of adequate protocols for propagation through tissue culture not only for the species but also for each cultivar.

The study was performed to find out whether factors such as the medium composition and explants influence the initiation process of *Lachenalia* 'Romaud' micropropagation. In the experiment four explant types were obtained from bulbs: inflorescence, stem, bulb scale and leaf. Explants were put on MS basic medium solidified with agar (0.7%), containing 3% of sucrose and supplemented with growth substances: 2.5 μM BA and 0.5 μM NAA or 0.5 μM BA and 2.5 μM NAA. After 8 weeks all explants were evaluated and scored for adventitious buds, bulbs and roots formation.

Analyzing all factors, the highest coefficient of bud and bulb formation was obtained respectively from scales and leaves on a medium containing a higher concentration of BA. Bulb scales and stems put on the medium with a predominance of BA did not produce any roots but the highest mean number of roots was obtained from stem and leaf explants grown on the medium supplemented with a higher concentration of NAA. Irrespectively of the medium composition, the same number of buds was obtain from all explants types. Moreover, the highest number of bulbs was observed on leaf explants and the lowest number of roots on scale explants. Irrespectively of the explant types, highest concentration of NAA resulted in producing lower number of buds and bulbs but favored the formation of roots.

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Multiplication and death of oogonia in developing and adult ovaries in the grass frog *Rana temporaria*

Magdalena Chmielewska, Beata Rozenblut-Kościsty, Maria Ogielska

Department of Evolutionary Biology and Conservation of Vertebrates, Institute of Environmental Biology, University of Wrocław, ul. Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: magdalena.chmielewska@uni.wroc.pl, beata.rozenblut@uni.wroc.pl, maria.ogielska@uni.wroc.pl

During ovarian development in anuran amphibians, mitotic activity of primary oogonia gives rise to the great number of diplotene oocytes. Our previous studies have shown that the final number of diplotene oocytes in a virgin-adult female is sufficient for all breeding seasons during her lifespan. Oogonia in adult females remain mitotically active in germ patches, but they soon degenerate and thereby do not replenish a the stockpile of diplotene oocytes (Ogielska and Kotusz, 2004; Ogielska et. al., 2013). The aim of the present study was to investigate fates of oogonial cells in developing and adult ovaries, and check a possibility of new diplotene oocytes formation after spawning eggs.

To check a possible *de novo* formation of diplotene oocytes from mitotically active oogonia in juvenile and sexually mature females, we tracked incorporation of 5-Bromo-2'-deoxyuridine (BrdU) during DNA synthesis in S-phase of oogonial cells. Four weeks after subcutaneous injection of BrdU, animals were sacrificed and BrdU expression and tissue localization was examined using immunohistochemical method and light and confocal microscopy. In sexually mature females we have not observed BrdU signal in diplotene oocytes. To further investigate the fate of oogonia and oocytes we examined degenerative processes leading to apoptosis. The expression of active caspase-3 was studied by immunofluorescent microscopy revealing apoptotic changes in small diplotene cells in 1–2 year old juveniles, which supports previously reported massive depletion of oocyte pool in developing gonads. In adult females, apoptosis was detected in degenerating oogonia and meiocytes as well as in previtellogenic diplotene oocytes.

The results obtained in this study are in accordance with our previous reports and confirm that oogonial cells in adult females of grass frog can multiply, but are unable to transform into the diplotene cells.

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Regulation of endogenous contractile activity of *Tenebriomolitor oviduct* by biologically active compounds

Szymon Chowański, Jan Lubawy, Grzegorz Rosiński

Department of Animal Physiology and Development, Adam Mickiewicz University in Poznań, Poland, e-mail: j.lubawy@amu.edu.pl, szyymon@amu.edu.pl

Insects form the largest and most diverse group of animals on the globe, representing over half of all known animal species. They are present in almost all habitats on the planet Earth. By many, insects are perceived as pests, which cause great loss in crops, or are vectors for many pathogens such as in example malaria. On the other hand, many insect species play positive role in economy. A good example is *Apismelifera* or *Bombyxmori* that are used by humans.

Because of the important role of insects in human economy, positive as well as negative, precise understanding of mechanisms responsible for regulation of breeding and development of this group of animals is very important. The activity of insect reproductive system is regulated by many factors, such as neurohormones or biogenic amines. These compounds may affect inter alia spermatogenesis, the processes of oocytes maturation in ovarioles, as well they can be involved in regulation of endogenic contractile activity of oviduct and ejaculatory duct, which may be transferred into the number of laid eggs, and finally decide about reproductive success.

In presented study we analysed the effect of two neuropeptides from allatostatin family, Dippu-AST1 and Grybi-AST B1 as well as regulators of muscarinic cholinergic receptors, agonist – karbachol and antagonists – scopolamine and atropine on endogenous contractile activity of *Tenebriomolitor* beetle oviduct.

Clearing technique for the study of embryo sacs in interspecific *Allium* hybrids

Alicja Chuda, Agnieszka Kiełkowska, Adela Adamus

Institute of Plant Biology and Biotechnology, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Cracow, Poland, e-mail: a.chuda@ogr.ur.krakow.pl

In this study clearing technique was applied to investigate the development of female gametophyte in two populations of interspecific hybrids: F_1 *A. galanthum* × *A. cepa* and F_1 *A. cepa* × *A. roylei* as well as in *Allium cepa* plants. Flower buds of each plant were fixed in FAA and stored in 70% ethanol. The ovules were isolated from the ovaries and cleared in methyl salicylate according to a procedure described by Mól (1988) with minor modifications. Cleared ovules were examined with a AxioImager.M2 microscope fitted with Nomarski interference contrast optics. Histological analysis showed that most of the observed embryo sacs of *Allium cepa* plants were typical and consisted of the egg apparatus with highly vacuolated egg cell and two synergids at the micropylar pole, central cell with two polar nuclei, and three antipodal cells at the chalazal pole. Both in F_1 *A. galanthum* × *A. cepa* and F_1 *A. cepa* × *A. roylei* hybrids disturbances in ovule and embryo sac structure were observed. In ovules mostly degeneration of nucellus was identified. In embryo sacs untypical polarization and cellularization occurred. Also abnormalities in vacuolization of egg apparatus cells were observed. In the studied hybrids degenearation of whole embryo sacs appeared frequently. The applied clearing technique was useful for the analysis of embryo sacs in interspecific *Allium* hybrids.

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Evaluation of phylogenetic signal in characters of a female reproductive system of clitellates (Annelida: Clitellata)

Joanna M. Cichocka[1], Aleksander Bielecki[1], Piotr Świątek[2], Anna Z. Urbisz[2], Dorota Pikuła[1], Iwona Jeleń[1]

[1]*Department of Zoology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland, e-mail: joanna.cichocka@uwm.edu.pl, alekb@uwm.edu.pl, dorota.pikula@uwm.edu.pl, iwona.jelen@uwm.edu.pl*
[2]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl, anna.urbisz@us.edu.pl*

Recently, features of the female reproductive system proved to be useful for reconstruction of leech (Hirudinida) phylogeny at the family level (Bielecki et al., 2013). The aim of this study was to evaluate the phylogenetic signal in these characters and accuracy of obtained phylogeny for expanded set of clitellate taxa. Parsimony analyses included representatives of 12 families of Clitellata. Character sets consisted of 22 features of the female reproductive system including ultrastructural ones and 25 intersubjectively selected morphological characters. Phylogenetic analyses were performed in PAUP* (Swofford, 2002) under a branch-and-bound search with TBR branch swapping. Accuracy of obtained phylogenies was evaluated by the numbers of: informative characters, equally parsimonious trees, nodes with bootstrap values greater than 50% and phylogenetic signal. Three indices were estimated for phylogenetic signal: the consistency index (CI), the skewness index (g1) and the data decisiveness index (DD). Parsimony analyses based on both the features of the female reproductive system separately and in combination with other morphological characters resulted in well supported phylogenies. All characters proved to be parsimony-informative. The number of most-parsimonious trees was much fewer in case of analyses based on female characters only and combined data set than in the analysis using other morphological features. However, the consensus cladogram based of female features was worse resolved and the number of clades supported by significant bootstrap values was lower comparing with the consensus tree generated with morphological characters. Indices of phylogenetic signal were high for all character sets, and the features of female reproductive system were characterized by the highest values of CI and DD. Our study demonstrated that the female reproductive characters for Clitellata do contain significant phylogenetic information.

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The embryo-suspensor in *Sedum atratum* L. (Crassulaceae): A developmental study using light and electron microscopy

Daria Czaplejewicz, Małgorzata Kozieradzka-Kiszkurno

Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, e-mail: daria.czaplejewicz@biol.ug.edu.pl, malgorzata.kozieradzka@biol.ug.edu.pl

The suspensor is a specialized, short-lived structure which functions mainly to position the embryo in the embryo sac and to supply the embryo with nutrients during the early stages of development (Kozieradzka-Kiszkurno et. al., 2011; Schwartz et. al., 1997). The development of the embryo suspensor in *Sedum atratum* L. was investigated using cytochemical methods, light and electron microscopy. Fully development and functioning suspensor consist of a large basal cell and a few chalazal cells arranged in two layers. The basal cell forms an anucleate micropylar haustorium. The micropylar haustorium gradually penetrates of both the funiculus tissue and the integument tissue up to chalazal parts of the seed. The walls of the haustorium and the micropylar part of the basal cell form prominent wall ingrowths which are covered by a plasma membrane and are typical for a transfer cells. The cytoplasm of the basal cell is rich in different organelles: mitochondria, plastids, dictyosomes, profiles of endoplasmic reticulum and vacuoles. Cytochemical tests showed presence of high amounts of macromolecules such as proteins, insoluble polysaccharides and lipids. Analysis of the development, cytochemical results and ultrastructure of the suspensor *S. atratum* suggests that it functioning in absorption and short-distance translocation of metabolites essential for growth embryo-proper.

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Effects of short peptides Led-NPF-1 and *Neb*-TMOF on the vitellogenesis of *Tenebrio molitor* beetle

Elżbieta Czarniewska[1], Mariola Kuczer[2], Grzegorz Rosiński[1]

[1]*Department of Animal Physiology and Development, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland, e-mail: czarniew@amu.edu.pl, rosin@amu.edu.pl*
[2]*Faculty of Chemistry, Wrocław University, Joliot-Curie 14, 50-383 Wrocław, Poland, e-mail: mariola.kuczer@chem.uni.wroc.pl*

In insect the gonadotropic hormones isolated from brain and ovaries show different activities. These hormones stimulate or inhibit ovary development and oocyte growth, affect of vitellogenin biosynthesis in the fat body, inhibit the synthesis of trypsin and serine proteases in the gut and they regulate the ecdysone biosynthesis in the follicular epithelium of the oocyte chamber (Czarniewska, 2013).

We tested *Neb*-TMOF and Led-NPF1 for an effect on ovaries of *Tenebrio molitor* females during their first reproductive cycle. Peptide hormone *Neb*-TMOF, originally isolated from the *Neobellieria bulata* ovaries, decreasing synthesis trypsin or trypsin-like enzymes in the midgut, inhibits the growth and maturation of oocytes in the fly ovary. Recent studies confirmed gonadoinhibitory activity of *Neb*-TMOF in two species of beetles *Tenebrio molitor* and *Zophobas atratus*. Other peptide hormone Led-NPF-1, isolated from the brain of *Locusta migratoria*, regulates gonadotropic cycle by stimulating the maturation of oocytes in the ovary of the insect. The mechanism of gonadotropic action of Led-NPF-1 is not known to date and suggests that it may rely on the induction of synthesis of JH and ecdysone, as well as stimulating the release of parsins in the *corpora cardiac* (Czarniewska, 2013).

We have shown that injection of nanomolar doses of *Neb*-TMOF caused induction of caspase activity both in germarium and vitellarium, what resulted in atresia of terminal follicle in *T. molitor* ovary. On the other hand, Led-NPF1 injection did not activate caspase but strongly stimulated intercellular space formation (patency) in follicular epithelium and accelerated deposition of vitellin in terminal follicle of *T. molitor*.

The results indicate the complex and pleiotropic regulation by peptide hormones gonadotropic cycle in insects.

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Can salinity of the Baltic Sea limit the embryonic development of the nematode *Contracaecum rudolphii*?

Janina Dziekońska-Rynko[1], Katarzyna Mierzejewska[2]

[1]*Department of Zoology, Faculty of Biology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-957 Olsztyn, Poland, e-mail: jdr@uwm.edu.pl*
[2]*Department of Fish Biology and Pisciculture, Faculty of Environmental Sciences, University of Warmia and Mazury in Olsztyn, Oczapowskiego 2, 10-719 Olsztyn, Poland, e-mail: katarzyna.mierzejewska@uwm.edu.pl*

Embryonic development of most nematodes proceeds in the natural environment. Eggs of soil nematodes, the so-called "geohelminths", are coated with a thick, three-layer shells that protects them against detrimental environmental factors. Eggs of nematodes belonging to the family Anisakidae (*Anisakis simplex*, *C. rudolphii*) are classified as "thin-shells" eggs, and during an embryonic development they display a high susceptibility to abiotic factors as temperature, water oxygenation and the salinity. The objective of the present study was to investigate, the effect of water salinity on the embryonic development of *C. rudolphii* under laboratory conditions. Nematode eggs were removed from the terminal section of the uterus of an adult females and placed in 0.9% and 3% NaCl, at the room temperature. Cultures dishes were aerated each day by a rapid filling up and emptying a pipette ended with an ejector. The progress of the egg development in each solutions was daily checked by examining the eggs under a Biolar compound microscope at 20 × 12.5 magnification. No egg development was observed when the culture was established; about 2% of eggs showed blastomere stage 2 only. On the second day, all the eggs reached the blastula stage. On the third day the late gastrula stage was recorded, whereas on the fourth day most of the eggs (70%) contained moulted larvae, which shed their cuticle inside the egg. The second-stage larvae (L2) moulted inside the egg, and the three-stage larvae (L3) hatched surrounded by a loose-fitting cuticle of the second moult. The results of this study indicate, that the nematode *C. rudolphii* may develop with the success both in fresh and salty waters. The experiment demonstrates the feasibility of embryonic development in waters with the salinity which corresponds to this of the coastal zones of the Baltic Sea (0.2–1.2%) as well as the Mediterranean and the Azov Seas, where the larvae of the nematode are relatively frequently detected in different fish species.

Evaluation of periostin in myocardial infarction in a mouse model – preliminary results of microarray screening

Dawid Fil, Wiesława Niklińska, Zygmunt Mackiewicz

Department of Histology and Embrology, Medical University of Bialystok, Kilinskiego 1, 15-269 Bialystok, Poland, e-mail: daveblue7@wp.pl

Introduction. Myocardial infarction (MI) is still one of the most common causes of heart failure, disease that impairing daily activity and aggravating in a significant way the quality of life of patients.Therefore, studies aimed at elucidating the molecular mechanisms involved in correct repair processes in damaged myocardium infarction have important practical aspect, because it may give rise to the search for new therapeutic strategies aimed at eliminating the effects of post-MI damage.One of the factors that play an important role in myocardial regeneration is a periostin protein encoded by the gene POSTN. The expression of this protein was abundant in the infarct border of human and mouse heart with MI. As shown by recent research extracellular periostin induced reentry of differentiated mammalian cardiomyocytes into the cell cycle.

Aim. The aim of this study was to show preliminary evaluation and comparisionof periostin in mice after myocardial infarction operation (MI) and mice after Sham-operation (Sham) using microarray screening method.

Material and methods. The study was conducted on 40 mice of 4-month-old male of reference wild-type C57BL /6. In general anesthesia with isoflurane maintained by inhalation, in 20 mice a surgery left coronary artery ligation (MI-operation) was performed and in the other group of remaining 20 – apparent operation (Sham-operation). After 16 weeks the animals were killed by cervical dislocation, and the obtainedheartswere provided to tubes and frozen.Then from each group (MI and Sham) 5 slices of the left ventricles of hearts were collectedand RNA isolation and pooling in their groups were performed for screening using Agilent 44K microarray. After obtaining results abioinformatic and statistical analysis were made using Agilent programs.

Results. The obtained preliminary results of the microarray screening in mouse cardiac tissue after MI have shown almost 7-times (FC = +6.865) more of periostincompared to Sham, which is correct for MI and during myocardium regeneration. As the global research show that periostin activates the integrins located in the cardiomyocytecell membrane such as αv, β_1, β_3 and β_5, in our screening results in group of mice after MI was also obtained several times more of integrin β_1 (FC= +5.071) and $\beta 3$ (FC= +3.048) compared to Sham, which confirms the activation of the necessary factors and begin the process of repair after MI.

Conclusions. Our obtained preliminary results confirm the hypothesis that periostin and activated by it integrin play an important role in the repair of the infarcted heart.The essential differences between the mice after MI and without it induce us to continue and extend the research in terms of quantity and quality of the periostin and other conditioned by it factors of repair of the heart.

Vesicular transport of a material originated from intranuclear bodies in oocytes of *Neocaridina davidi* (Crustacea: Malacostraca: Decapoda)

Arnold Garbiec

Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, 21 Sienkiewicza Street, 50-335, Wroclaw, Poland, e-mail: arnold.garbiec@uni.wroc.pl

In Crustacea the oocytes develop either with or without the support of their sibling germ cells, the nurse cells (trophocytes). In *Neocaridina davidi*, a representative of Decapoda, the oocytes are the only germline cells within the ovaries. The somatic cells of the ovary are poorly developed and surround individual oocytes. The growth of the oocytes relies mainly on the oocyte activity. At the beginning of the oocyte growth (previtellogenesis) the germinal vesicle (oocyte nucleus) is centrally located and irregular in shape. In the oocyte cytoplasm (ooplasm) a few organelles such as mitochondria, dictyosomes and endoplasmic reticulum components are visible. With the progress of previtellogenesis both the oocyte nucleus and the ooplasm structure changes dramatically. Within the nucleus an increasing amount of vesicles neighboring the inner nuclear membrane are observed. In more advanced previtellogenesis the vesicle associate with various nucleus domains including nucleolus and within their lumen granules of electron-dense material appear. Meanwhile, the number of organelles within the cytoplasm significantly increases. In the close vicinity of the germinal vesicle the ooplasm is enriched with small perinuclear granules as well as a few endoplasmic reticulum elements. In some distance from the nucleus the cytoplasm is rich in numerous cisterns with electron dense granules, some of these cisterns remain in the vicinity of dictyosomes. The appearance of the intranuclear vesicles in the oocytes of *N. davidi*, and their association with nuclear domains strongly suggest that they contribute to the very intense nucleocytoplasmic transport, however, the identity of molecules transported by this unique mechanisms awaits further studies.

Ovary organization in *Propappus volki* (Clitellata, Propappidae) – preliminary results

Szymon Gorgoń, Piotr Świątek

Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: szymon.gorgon88@gmail.com, piotr.swiatek@us.edu.pl

Propappus volki, a freshwater oligochaetous annelid, represents the family Propappidae, although *Propappus* was initially regarded as a member of family Enchytraeidae. Our preliminary studies revealed that in the specimens of *P. volki* that have a well-developed clitellum, the paired ovaries are long and have an S-like shape. The ovaries are composed of germ-line cysts of interconnected cells and somatic cells that form the gonad envelope. The organization of the cyst is broadly the same as in other clitellate annelids that have been studied to date, i.e. each germ cell in a cyst is connected with the central nuclei-free cytoplasm (cytophore) via one intercellular bridge. The ovaries are polarized, i.e. there is a visible gradient of germ cell development along the long axis of the ovary. There are numerous cysts formed of cells in meiotic prophase (pachytene) within the slightly narrower proximal end of the ovary. The cytophore in such cysts is extremely thin and has the form of thin cytoplasmic strands that are lined by an electron-dense rim. The middle part of the ovary is occupied by numerous cells of an uniform morphology. These cells are connected to the well-developed cytophore. Within the outermost part of the ovary (distal end), which extends freely into the segmental cavity, two cell categories can be distinguished: 1) larger cells that accumulate cell organelles and the yolk are future egg cells and 2) more numerous, but not growing cells, which will probably become nurse cells. The early vitellogenic oocytes detach from the ovary and float freely in the body cavity where they continue to accumulate reserve material.

Our results show that the organization of the gonad in *P. volki* is substantially different than in *Enchytraeus albidus* (Enchytraeidae) and favor the establishment of the family Propappidae.

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Calcium-binding proteins immunoreactivity in the preoptic area of the guinea pig (*Cavia porcellus*) during prenatal development

Beata Hermanowicz, Krystyna Bogus-Nowakowska, Witold Żakowski, Anna Robak

Department of Comparative Anatomy, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, pl. Łódzki 3, 10-727 Olsztyn, Poland, e-mail: beata.hermanowicz@uwm.edu.pl

Calcium ions (Ca^{2+}) play an important role in neuronal functioning, including neurotransmitter release or regulation of neuronal plasticity. The calcium-binding proteins (CaBPs): calbindin (CB) and calretinin (CR) can be used as cellular markers in neuroanatomical research and are involved in maintaining intracellular calcium ion homeostasis (Andressen et al., 1993). The aim of this study was to evaluate the pattern of distribution of CB- and CR- immunoreactive (-ir) elements in the preoptic area (POA) during prenatal development of the guinea pig. Preoptic area participates in the regulation of many physiological processes, for example regulation of maternal behavior (Pereira and Morrell, 2009). Animals brains from the 40th, 50th and 60th embryonic day (E40, E50, E60) were used in the study. A routine single-labelling immunofluorescence was applied to visualize CB- and CR-immunoreactivity. The CB- and CR- ir structures were observed at each examined fetal stages of the preoptic area in the guinea pig. Among these stages, the highest immunoreactivity for both studied proteins seems to be at the 60th embryonic day. In the POA in all studied stages the density of calbindin-ir cells was higher than calretinin-ir cells. The most numerous CB- and CR-ir perikarya were seen in the periventricular preoptic nucleus of the POA at E60. In general, CB-ir perikarya were singly spaced but some of them occurred in clusters. They were round, oval, sometimes fusiform or triangular, whereas calretinin containing perikarya were mostly round and only few of them were triangular.

The present study indicates that CB shows higher immunoreactvity than CR in the preoptic area of the guinea pig and CB- immunoreactivity is the highest at 60th day of prenatal development. This may suggest that calbindin may be involved in the participation of the essential regulatory functions in this region during prenatal development in the guinea pig, especially before the birth.

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Comparative morphology of the olfactory organs of selected gobies species (Gobiidae, Pisces)

Bartosz Holecko[1], Krystyna Żuwała[1], Michał Kuciel[2], Joanna Grabowska[3], Bartłomiej Arciszewski[4]

[1]*Department of Comparative Anatomy, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: krystyna.zuwala@uj.edu.pl*
[2]*Poison Information Centre, Clinic of Toxicology Collegium Medicum, Śniadeckich 10, 31-531 Cracow, Poland*
[3]*Department of Ecology and Vertebrate Zoology, University of Łódź, Pilarskiego 14/16, 90-231 Łódź, Poland*
[4]*Hel Marine Station, Institute of Oceanography, University of Gdańsk, Morska 2, 84-150 Hel, Poland*

The present study describes the morphology of the olfactory organ in three teleost species from the Gobiidae Family: *Neogobius fluviatilis*, *Neogobius melanostomus* and *Proterorhinus semilunaris*. Studies were performed using standard methods of scanning electron microscopy and light microscopy (SEM, LM). The main feature of the olfactory organ of these three species that distinguishing them from most of the teleosts described to date is the absence of the olfactory rosette and decisive extension of the olfactory chamber. Similar features have been noted previously in *N. melanostomus* (Belanger et al., 2003) and in several representatives of the Oxudercinae subfamily (Kuciel et al., 2011, 2013). Tubular inlet nostrils in *N. fluviatilis* and *N. melanostomus* are located in the front part of the head above the upper lip. In contrast in *P. semilunaris*, a tubular inlet nostril is located on the upper lip. There is also a tubular-like outlet nostril in the pre-orbital part of the head. In fish of the *Neogobius* genus, the olfactory chamber is oval in shape. In *P. semilunaris* it forms a channel. Olfactosensory epithelium in all three species is present in the olfactory chamber/channel bottom, and in *P. semilunaris* and *N. fluviatilis* it is present also on the side walls of the chamber. In all studied species, accessory nasal sacs were found which act as a mechanical part of the olfactory organ. In *N. melanostomus* there are two accessory nasal sacs and one accessory nasal sac in *N. fluviatilis* and *P. semilunaris*, a rare arrangement in Perciformes.

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Relationship between the storage bodies and the oogenesis in *Isohypsibius granulifer granulifer* (Tardigrada: Eutardigrada)

Marta Hyra, Marcin Deperas, Michalina Kszuk-Jendrysik, Agnieszka Włodarczyk, Lidia Sonakowska, Magdalena Rost-Roszkowska, Izabela Poprawa

Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: martah1988@o2.pl

The storage bodies (storage cells, body cavity cells, speicher cells) are only cells that move passively in the body cavity of tardigrade. Their main function is to store the food reserves. The material for the culture was gathered from the pond in the Botanical Garden of Jagiellonian University in Cracow (Poland). The ultrastructural changes of the storage cells during oogenesis in *Isohypsibius granulifer granulifer* (Tardigrada: Eutardigrada) were analyzed using light and transmission electron microscopes, and some histochemical methods. During process of oogenesis ultrastructure of the storage cells shows their intense metabolic activity. They have large nuclei, well-developed cisterns of RER, ribosomes, mitochondria, Golgi complexes and nonhomogenous spheres of the reserve material. After oviposition these cells are small and their divisions are observed sporadically. The amount of the reserve material accumulated in the storage cells, changes during the process of oogenesis. The amount of the reserve material is small during previtellogenesis, increases during early and middle vitellogenesis and starts to decrease at the end of vitellogenesis. After the eggs laying, the cells have a small amount of the reserve material. It suggest that in *I. g. granulifer*, similar to other tardigrades (Szymańska, 1994, Poprawa, 2006), storage cells can take part in the synthesis of yolk precursors.

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Development of the stomach in the domestic goose (*Anser anser f. domestica*) from 9th to 25th day of incubation

Hanna Jackowiak, Izabela Kmiecik,
Kinga Skieresz-Szewczyk, Ewelina Prozorowska

Department of Histology and Embryology, Faculty of Animal Breeding and Biology, Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznan, Poland, e-mail: hannaj@up.poznan.pl

The stomach in birds consists of two parts: proventriculus (glandular stomach) and ventriculus (muscular stomach). The aim of the study was to follow the developmental rate of both chamber of stomach of goose and characterize histogenesis of the their wall.

The study is proceed on the domestic goose embryos from 9th to 25th day of incubation by using LM and SEM methods. The studies were conducted with approval of Ethical committee.

During the incubation the intensive growth of stomach was observed. The weight of the stomach increase about 173 times and the length increase 11 times. It change also the proportion of size the two-chambered stomach. Until the 13th day proventriculus it is longer chamber of stomach, but before hatching the decrease of the length to the half part of the ventriculus was observed. The development of the wall of the stomach occurs in two periods. The first – embryonic period lasts between 9th to 15th day and the second – differentiation period lasts from 16th to 25th day. In the first period the embryonic tissues transform into mucosa layer with primordia of proper glands, muscular and serosa layer. During the differentiation period glandular primordia in the proventriculus and ventriculus develop. Proper glands of the proventriculus transform from simple tubes into compound glands from 19th day of incubation. Between 19th and 23rd day they produce neutral glycoproteins and from 24th day acid glycoproteins. Simple ventriculus glands start to produce neutral glycoproteins from 16th to 18th day and on 24th – 25th day elongate and reach the muscular layer.

At the end of incubation the both chamber of geese's stomach is ready to fulfill the function as in the adult birds. The development of the of superficial glands in proventriculus and glands of ventriculus will continue after hatching under the influence of the collected food.

SEM observations on embryonic development of the tongue in *Lacerta agilis* (Reptilia)

Hanna Jackowiak[1], Weronika Rupik[2],
Kinga Skieresz-Szewczyk[1], Elwira Swadźba[2],
Ewelina Prozorowska[1]

[1]*Department of Histology and Embryology, Faculty of Animal Breeding and Biology, Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznan, Poland, e-mail: hannaj@up.poznan.pl*
[2]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland*

The tongues in adult sand lizard have characteristic morphology with divided apex of the tongue covered by keratinized epithelium and numerous mucosal fold on dorsal and lateral surfaces of tongue, called lingual papillae. The aim of the SEM studies was to analyze the morphogenesis of the tongue in sand lizard embryos from 10–30 day of incubation.

The mandibles with tongues were fixed in Karnovsky solution, dehydrated and critical point dried. Finally the samples were covered with gold and observed in scanning electron microscope ZEISS 435 VP.

The tongues of very early embryo on 10–12 day of incubation have triangular shape with slightly furrow on the apex as primordium of future anterior processes. The mucosa on dorsal surface of tongue is flat with embryonic epithelium. Between 14–19 day of incubation the apical furrow get deeper. In posterior part of the tongue at laryngeal entrance the mucosa is slightly folded. Between 21 and 23 day of incubation the anterior processes elongate and are covered partly by keratinized epithelium. The dorsal and lateral mucosa on body and root of the tongue shows delicate pattern of parallel ridges running transversely to the main axis of tongue. On 26 –30 day of incubation these mucosal ridges are observed only on lateral borders of the tongue. In the middle surface of tongue mucosal ridges divided into smaller squamae, directed to posterior part of tongue. The measurement of tongues during incubation showed in 2.5× increase in length and also in width.

The SEM observation showed that the development of microstructures of the tongue in sand lizard are continued after hatching.

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).

Study of megasporogenesis and female gametophyte formation in *Taraxacum belorussicum* Val. N. Tikhom. (sect. *Palustria*) using Nomarski DIC optics

Agnieszka Janas, Krystyna Musiał, Maria Kościńska-Pająk

Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: aga.janas90@gmail.com, k.musial@uj.edu.pl, maria.pajak@uj.edu.pl

The genus *Taraxacum* (Asteraceae) represents a polyploid complex in which the ploidy level is linked to the mode of reproduction: diploid species reproduce sexually, whereas polyploids are obligate or facultative apomicts. So far, out of 373 species of dandelions recognized in Poland, only a few were embryologically analysed on paraffin microtome sections.

The aim of our investigations was to examine the embryological processes in the ovules of *Taraxacum belorussicum* from section *Palustria*. In Poland, dandelions representing this section are rare, poorly explored, and endangered because of human activity causing degradation of their natural habitats. In the present study we applied a less time-consuming clearing tissue technique with the use of methyl salicylate. The whole cleared ovaries were examined with Nomarski optics (DIC microscopy). The use of this technique facilitates not only large-scale embryological observations but also provides high-quality images.

The reproduction trace of *T. belorussicum* involves meiotic diplospory. A single archesporial cell differentiates at the micropylar pole of the anatropous, tenuinucellate ovule with one integument. After the first altered meiotic division, a restitution nucleus is formed. The second meiotic division is regular and leads to the formation of two unreduced megaspores (diplodyad). The chalazal cell of diplodyad acts as a functional megaspore. The subsequent three mitotic divisions led to the formation of an unreduced female gametophyte whose structure corresponds to the *Polygonum* type.

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Balbiani bodies in oocytes of a spider, *Nuctenea* sp. and a harvestman, *Phalangium opillio* share some similarities in the structure and behavior

Izabela Jędrzejowska

Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Sienkiewicza 21, 50-335, Wroclaw, Poland, e-mail: izabela.jedrzejowska@uni.wroc.pl

Oocytes growth proceeds in two consecutive stages: previtellogenesis and vitellogenesis. During previtellogenesis oocytes develop and accumulate a great number of organelles and macromolecules (RNAs and proteins), whereas in vitellogenesis storage of reserve material (yolk, lipids and glycogen) in the oocyte cytoplasm (ooplasm) takes place. One of the most spectacular event of previtellogenic growth is an appearance in the ooplasm an organelles assemblage, referred to as the Balbiani body. The Balbiani body appears in the close vicinity of the oocyte nucleus, usually at its one pole, what breaks the oocyte symmetry. It has been evidenced that in some animals like e.g. insects and amphibians, the Balbiani body consists of a variety of organelles including mitochondria, endoplasmic reticulum, dictyosomes, multivesicular bodies, cytoskeleton elements and macromolecules (RNAs and proteins). Comparative studies concerning the Balbiani body functions indicate that the Balbiani bodies are involved in e.g. germline determination, patterning of the embryo and selection of healthiest mitochondria, however, the definitive functions of Balbiani bodies remain elusive.

Among arachnids the Balbiani body is a commonly found structure of the growing oocytes. However, the composition and behavior of the Balbiani bodies in arachnids are not stable features. Analysis of the ooplasm organization in two distantly related arachnids, namely the harvestman, *Phalangium opilio* (Arachnida, Opiliones) and the spider, *Nuctenea* sp. (Arachnida, Araneidae) revealed that in these arachnids the Balbiani bodies are cytoplasmic collections of a simple structure. A prevailing component of the Balbiani body is the nuage (RNA-rich) material that appears around the nucleus in early previtellogenic stages. In both investigated arachnids during previtellogenesis the Balbiani body translocates from the juxtanuclear position to the cortical ooplasm where it eventually disperses. A detailed ultrastructural analysis showed that in *Phalangium* in addition to the nuage material the Balbiani body contains a small amount of vesicles, whereas in *Nuctenea*, the Balbiani is a complex of the nuage material and mitochondria.

The results of this study strongly indicate that in *Phalangium* and *Nuctenea* the Balbiani bodies are structures involved in RNAs storage and transportation within the ooplasm. It is also tempting to speculate that within the Balbiani bodies RNA modifications take place.

Tetraploid *Cobitis* (Pisces, Cobitidae) males – a histological description of the testes

Dorota Juchno[1], Alicja Boroń[1], Krzysztof Grucza[1], Anna Leska[1], Olga Jabłońska[1], Anna Przybył[1], Anna Pecio[2], Beata Cejko[3], Radosław Kowalski[3]

[1]*Department of Zoology, University of Warmia and Mazury in Olsztyn, Oczapowski 5, 10-918, Poland, e-mail: juchno@uwm.edu.pl*
[2]*Department of Comparative Anatomy, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland*
[3]*Department of Gamete and Embryo Biology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland*

The diploid-polyploid complexes of *Cobitis* distributed in Poland are usually composed of one 2n parental species, *C. taenia* or *C. elongatoides*, triploid hybrid females and intriguingly tetraploid hybrids of both sexes. Tetraploid *Cobitis* females attain maturity and produce mature eggs but 4n *Cobitis* males have not been able taking part in reproduction, even to stimulation the gynogenetic eggs of 3n females to development. Thus, the role of 4n males in reproduction within complex is not clear. The aim of this study was to describe histology of testes of tetraploid *Cobitis* hybrids from the other population.

Reproductive capacity was investigated in 2n *C. taenia* and 4n Cobitis males collected from May to October, from the Pilica and Kortówka Rivers. Testes were fixed with Bouin's or 4% formaldehyde solution; sections 5–7mm think were stained with Delafield's hematoxylin and eosin. The ploidy of all males was verified karyologically. Pectoral fin of all males bases a well-development *lamina circularis*, which manifested their maturation.

Microscopic examination of tetraploid males' testes indicates that they were sterile. Tubules contain cysts with a large number of spermatocytes I and II, and a less number of spermatogonia. No functional spermatozoa could be observed, but cells exhibiting pycnosis were frequently detected. These cells were smaller in sizes than spermatogonia and spermatocytes I, and their nuclei showed a range of over staining with hematoxylin in an irregular shape.

In the same time, *C. taenia* testes showed normal typical restricted lobular structure. During the reproductive season have been observed spermatogonia, spermatocytes, spermatids and spermatozoa in the testes of the 2n *C. taenia* males. We documented for the second time the lack of reproductive ability of 4n *Cobitis* males from the population not investigated formerly.

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Diplotene chromosomes of oocytes of polyploid hybrid *Cobitis* (Pisces, Cobitidae)

Dorota Juchno[1], Alicja Boroń[1], Aneta Spóz[1], Roman Kujawa[2], Justyna Kolczyńska[1]

[1]*Department of Zoology, University of Warmia and Mazury in Olsztyn, Oczapowski 5, 10-918, Poland, e-mail: juchno@uwm.edu.pl*
[2]*Department of Lake & River Fisheries, University of Warmia and Mazury in Olsztyn, Oczapowski 5, 10-918, Poland*

The hybridization of *Cobitis* species is connected with unisexual reproduction and with polyploidy. Diploid (2n=49) and triploid (3n=74) *Cobitis* hybrids reproduce clonally or semiclonally *via* gynogenesis or hybridogenesis. The 2n hybrid females produce unreduced mainly gynogenetically developing eggs, whereas 3n *Cobitis* females seem to produce triploid eggs that most of their offspring are triploid, but part of their eggs are fertilized and develop into tetraploids (4n=98). Females of the last one probably may lay diploid eggs.

The aim of this study was to estimate (for the first in regarding the *Cobitis* occurring in Poland) the meiotic chromosomes in the germinal vesicles (GVs) of mature oocytes of the triploid and tetraploid *Cobitis* loaches.

Cytological analyses of oocytes were made on two diploid, 15 triploid and two tetraploid females following the procedures of Itono et al. (2006). Ploidy status of all individuals was verified by chromosome counting. The females were injected with ovopel. Full-grown oocytes were incubated with the maturation inducing steroid 17α-20β dihydroxy-4-pregnen-3-one. The oocytes were periodically collected and fixed with acetic acid and the germinal vesicle was mechanically taken and fixed with Carnoy`s solution. The isolated GVs were stained with DAPI and meiotic chromosomes were observed under fluorescence microscope.

Germinal vesicles isolated from the *C. taenia* ovaries contained 24 bivalent chromosomes having chiasmata. So, each containing two pairs of homologues. In GVs of triploid and tetraploid females different stages of prophase I: zygoten, diploten and diakinesis were observed. The last two stages were observed most frequently, because of chromosomes condensation; the bivalents with chiasmata were visible. The majority of GVs isolated from the ovaries of triploid (3n=74) females contained 74 bivalents, but trivalents, univalents and other unusual meiotic configuration were not detected. In only two GVs of tetraploid (4n=98) females, 96 or 98 bivalents were detected but quadrivalents have not been observed. Summarizing, no reduction in the ploidy level during the formation of the *Cobitis* hybrids egg was observed.

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Two types of mitochondria in the early zygote of *Gagea lutea* – is there a biparental inheritance of mitochondria in this species?

Małgorzata Kapusta[1], Joanna Rojek[1], Monika Maciąg-Dorszyńska[2], Jerzy Bohdanowicz[1]

[1]*Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, e-mail: malgorzata.kapusta@biol.ug.edu.pl*
[2]*Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland*

Gagea lutea (Yellow Star of Betlehem) is a spring ephemeral, monocot plant of the lily family. *G. lutea* is a bulb geophyte distributed throughout Europe and grows mainly in shady habitats of deciduous woodland (Yoshie, 2008). Ultrastructural and immunocytochemical studies showed numerous ellipsoidal mitochondria in generative cells of *G. lutea*. Mitochondria had condensed conformation with dense matrix and numerous cristae. Unlike in male gametophyte, the egg cell mitochondria had orthodox conformation, spherical shape, few short cristae and electron translucent matrix with thin fibrils (likely mtDNA). During double fertilization, at the stage of postmitotic karyogamy when nuclei of egg cell and sperm cell were partially fused, these two morphologically different types of mitochondria were observed in cytoplasm of the early zygote. Moreover, in the chalazal region of the early zygote's cytoplasm occurred an enucleated cell body (ECB) surrounded by two membranes and containing dense material. Structures resembling male gametophyte mitochondria, ER and also autophagic vacuoles were noted inside the ECB. The existence of ECB in the early zygote cytoplasm may indicate sperm cell cytoplasm transmission into the egg cell during double fertilization (Mogensen, 1988).The organellar DNA of higher plants may be inherited maternally and less often paternally or biparentally (Nagata, 2010). In studied species we observed the selective increase of mitochondrial DNA in maturing generative cell, the presence of numerous mitochondria in sperm cells, two types of mitochondria and ECB in the early zygote. These cytological evidences may support the hypothesis of biparental inheritance of mitochondria in *Gagea lutea*.

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Isolation and differentiation of mesenchymal stromal cells from fetal membranes and human term placenta

Gabriela Kmiecik[1], Wiesława Niklińska[1], Danuta Lipińska[2], Paweł Kuć[3], Zygmunt Mackiewicz[1]

[1]*Department of Histology and Embryology, Medical University of Bialystok, Waszyngtona 13, 15-269 Bialystok, Poland, e-mail: zmzmackiewicz@gmail.com*
[2]*Department of Endocrinology, Diabetology and Internal Medicine, Medical University of Bialystok, Waszyngtona 13, 15-269 Bialystok, Poland*
[3]*Department of Perinatology, Medical University of Bialystok, Waszyngtona 13, 15-269 Bialystok, Poland*

Bone marrow (BM) is a traditional source of mesenchymal stromal cells (MSCs), a particular cell type capable to differentiate into mesodermal lineage cells. However collection of BM is associated with an invasive procedure and results in relatively small number of these cells. As an alternative to bone marrow, human term placenta and fetal membranes are considered to be potential source of MSCs.

Aim of the study. To confirm presence of MSCs within different parts of human term placenta and fetal membranes.

Material and methods. Human term placentas (n=3) were collected after caesarean section and processed immediately. Amnion and chorion were manually separated from each other, washed and subjected to enzymatic digestion. Amnion fragments were digested with trypsin-EDTA and collagenase, chorion fragments were with dispase and collagenase. The internal section of central placental lobules was dissected and digested with trypsin-EDTA, while umbilical cord MSCs were isolated by explant culture. Isolated cells were designated as: amnionic mesenchymal stromal cells (AMSCs), chorionic mesenchymal stromal cells (CMSCs), placental tissue derived cells (PTDCs) and umbilical cord mesenchymal stromal cells (UCMSCs). At passage 2 (P2) presence of CD90, CD105, CD73 and CD34 was assessed by flow cytometry. For adipogenic differentiation AMSCs, CMSCs and PTDCs at P2 were cultivated in adipogenic differentiation medium for 3 weeks and stained with Oil Red 0 dye to visualize lipid vacuoles.

Results. Cells were successfully isolated from amnion, chorion, umbilical cord and proper placental tissues from all 3 placentas. During cultivation, adherent fraction of cells displayed spindle-shaped morphology typical for MSCs. AMSCs, PTDCs, UCMSCs expressed high levels of CD90 and CD73, while CMSCs presented high level of CD73 and low of CD90. Expression of CD105 was moderate within cell types. The adipogenic ability of AMSCs, CMSCs and PTDCs was proved by presence of abundant lipid vacuoles, especially in PTDCs.

Conclusions. Cells isolated from amnion, chorion and proper placental tissue shared morphology and immunophenotype characreristic for MSCs and showed capacity of adipogenic differentiation. Human term placenta is readily accessible source of MSCs which present the potential to differentiate into mesoderm-derived cell lineage making them highly interesting in tissue engineering strategies.

Endosymbiotic bacteria in *Macrosteles laevis* (Insecta, Hemiptera, Cicadellidae: Deltocephalinae). Ultrastructure, distribution and transovarial transmission

Michał Kobiałka, Anna Michalik, Teresa Szklarzewicz

Department of Developmental Biology and Morphology of Invertebrates, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: michal.kobialka@uj.edu.pl, a.michalik@uj.edu.pl, teresa.szklarzewicz@uj.edu.pl

The presence of endosymbiotic bacteria in the insect body is widespread. Literature data show that in the body of about 20% of all species of insects the endosymbiotic microorganisms (bacteria or yeasts) are present. Endosymbionts are usually harbored in specialized cells termed bacteriocytes (=mycetocytes), but they can also be found in the ovaries, midgut, fat body and hemolymph as well. As a rule, bacteriocytes are grouped in large organs called bacteriomes (=mycetomes) which are located near ovaries. The presence of endosymbionts in the body of insects is necessary for their growth and development. It is generally known that endosymbiotic microorganisms are responsible for providing their hosts substances missing in the diet. Since hemipterans consume phloem sap deficient in essential amino acids, their endosymbionts are responsible for synthesis of missing substances. Endosymbiotic microorganisms receive from their hosts habitat and certain substances. Endosymbionts are transovarially (vertically) transmitted from one generation to the next. Ultrastructural studies on endosymbionts of *M. laevis* revealed that in the body of these hemipterans the giant bacteriomes are present. The bacteriomes are surrounded by a one-monolayered epithelium and contain three types of morphologically distinct bacteria. The peripheral bacteriocytes contain large, electron-dense pleomorphic bacteria. The centrally located bacteriocytes comprise large, electron-translucent pleomorphic bacteria. The cytoplasm of some electron-dense pleomorphic bacteria is tightly packed with small rod-shaped bacteria. All types of bacteria are transovarially transmitted from the mother to offspring. The microorganisms leave the bacteriocytes, transverse the follicular cells in the region of the posterior pole of the oocyte and enter the perivitelline space. Finally, they accumulate in a deep invagination of the oolemmma.

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Primary and secondary endosymbionts of psyllids (Insecta, Hemiptera: Psylloidea)

Marta Kot, Anna Michalik, Teresa Szklarzewicz

Department of Developmental Biology and Morphology of Invertebrates, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: marta.kot@uj.edu.pl

The endosymbionts of representatives of three families of psyllids: *Psyllopsis fraxinicola* (Liviidae), *Aphalara polygoni* (Aphalaridae), *Craspedolepta ommisa* (Aphalaridae), *Rhinocola aceris* (Aphalarariidae) and *Trioza urticae* (Triozidae) were investigated by light, fluorescence and electron microscopy. The ovaries of all examined psyllids are accompanied by huge organs termed bacteriomes. In the body of all examined species two lobated bacteriomes have been observed. Each bacteriome consists of numerous, spherical, uninucleate, polyploid bacteriocytes and a syncytial region. Both bacteriocytes and syncytial region are occupied by endosymbiotic microorganisms. All examined psyllids possess two kinds of morphologically distinct endosymbiotic microorganisms: the bacteriocyte-associated endosymbionts that are regarded as primary endosymbionts (P-symbionts) and the syncytium-associated endosymbionts that are regarded as secondary endosymbionts (S-symbionts). The histological observations have revealed that P-symbionts of psyllids: (1) are more numerous than S-symbionts, (2) have irregular shape, (3) are morphologically similar in different species of psyllids, (4) are present in all examined specimens and (5) occur only in the bacteriocytes. In contrast to P-symbionts, secondary endosymbionts are less numerous and are characterized by distinct shapes and sizes in different species of psyllids.

Both primary and secondary endosymbionts are transmitted from one generation to the next maternally (=vertically, transovarially). The process of the infection of ovaries by bacteria takes place in the older females. Before infection the P-symbionts change their shape from irregular into spherical. Then, both kinds of bacteria leave the bacteriomes and start to migrate towards the ovaries. In psyllids, terminal oocytes at the stage of advanced choriogenesis are infested. Endosymbionts enter the cytoplasm of follicular cells surrounding the posterior pole of the oocytes and assemble in a deep depression of the oolemma (=in the perivitelline space) in the form of a 'symbiont ball'.

Morphological, histological and ultrastructural features of osmophores and nectary of *Bulbophyllum wendlandianum* (Kraenzl.) Dammer as a representative of the section *Cirrhopetalum* Lindl. (Bulbophyllinae Schltr., Orchidaceae)

Agnieszka K. Kowalkowska,
Małgorzata Kozieradzka-Kiszkurno,
Sławomir Turzyński, Daria Czaplejewicz

Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, e-mail: gnieszka.kowalkowska@biol.ug.edu.pl

The representatives of section *Cirrhopetalum* are spread throughout the subtropical and tropical regions of mostly from the Old World towards Oceania. The characteristic features of these species are umbellate inflorescence and elongated lateral sepals. The species from Bulbophyllinae are generally regarded as fly pollinated: myiophilous or sapromyiophilous. Since enticement to sapromyiophilous flowers is frequently based on deception, the flies are attracted by the scents, colours and surfaces, which imitate fly's natural food sources or their brood sites. By means of the floral anatomical investigation (micromorphology, histochemistry, ultrastructure), we wanted to indicate places of secretory activity and the character of secreted substances.

The flowers of *Bulbophyllum wendlandianum* fulfill features that characterize sapromyiophilous flowers, such as floral colours, the presence of motile appendages and see-saw lip. The appendages of dorsal sepal and petals function as osmophores. The cells at anthesis were characterized by large vacuole and peripheral cytoplasm, which contained large nucleus, numerous rough endoplasmic reticulum, mitochondria, starchless plastids with plastoglobuli and intraplastidal membranes. The exudation was transported inside vesicles via granulocrine secretion. The cuticle proper seemed to be completely permeable, stretched and formed swellings on the entire cell surface, sometimes at the points between adjoining epidermal cells. On the surface of appendages, a few amount of lipids were present, with meagre accumulation. The nectary comprised a secretory epidermis and few subepidermal layers and was located in central groove on the adaxial surface. The dense cytoplasm contained abundant mitochondria, frequent fully developed dictyosomes, free ribosomes, lipid drops, multivacuolar body, myelin-like figures. In large vacuole tannin-like materials were detected. The vesicles building into plasmalemma were frequently noted, which could indicate transport of volatile/nectar components. The nectariferous epidermal cells were more intensively stained on proteins. These results need further confirmation with chemical analysis of different exudates produced on appendages and from lip groove.

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Immunocytochemical localization of factors related to chromatin silencing in male gametophyte cells in *Hyacinthus orientalis*

Marlena Kozłowska, Katarzyna Niedojadło, Marta Iwon, Elżbieta Bednarska-Kozakiewicz

Department of Cell Biology, Faculty of Biology and Environmental Protection, Nicolaus Copernicius University, Toruń, Poland, Lwowska 1, 87-100 Toruń, Poland, e-mail: markoz@doktorant.umk.pl

During maturation of pollen grains, silencing of transcriptional activity and condensation of chromatin in vegetative and generative cells are observed. However, in time of pollen tube growth reactivation of transcriptional activity takes place in both those cells [1;2].

Regulation of transcriptional activity depends on numerous groups of factors. At the current step of knowledge the causes of this complex process are searched in the epigenetic mechanisms.

Our investigation was carried out on male gametophyte cells of *Hyacinthus orientalis* – mature pollen grains and in vitro growing pollen tubes. The aim of this study was to localize factors related to inactive chromatin: 5-methyl cytosine (5meC) and histone deacetylase 1 (HDT1).

In mature pollen grains 5meC was localized in both nuclei but HDT1 could not be detected. In early stages of pollen tubes growth we observed progressive increase of the labeling with 5meC in the vegetative nucleus and 3 different patterns of signal distribution in the generative nucleus: (1) labeling was not detected, (2) fluorescence of the nucleus was comparable to the vegetative nucleus, (3) signal was present mainly as a ring on the border of the nucleus. After 12 hours of the pollen tube growth the signal from the 5meC was localized mainly around the generative nucleus. The signal revealing the occurrence of HDT1 was present in the nuclei after being transferred to the pollen tube. In the early stages of pollen tube growth we frequently observed signal in both generative and vegetative nuclei. In contrast, after 12 h pollen tube growth labeling with HDT1 was localized mainly in vegetative nucleus and tube cytoplasm in the form of aggregates.

Those analyses suggest that the changes in chromatin condensation and transcriptional activity in the generative and vegetative nuclei during pollen tube growth could be a result of epigenetic modifications.

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The ultrastructure of the midgut epithelium of *Telodeinopus aoutii* (Myriapoda, Diplopoda)

Michalina Kszuk-Jendrysik[1], Agnieszka Sosinka[1], Magdalena Rost-Roszkowska[1], Izabela Poprawa[1], Marta Hyra[1], Lidia Sonakowska[1], Karolina Kamińska[1], Jitka Vilimova[2], Karel Tajovsky[3]

[1]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: michalina_kszuk@o2.pl, magdalena.rost-roszkowska@o2.pl, izabela.poprawa@us.edu.pl*
[2]*Faculty of Science, Department of Zoology, Charles University, Vinicna 7, 128 44 Prague 2, Czech Republic, e-mail: vilim@natur.cuni.cz*
[3]*Institute of Soil Biology, Biology Centre AS CR, Na Sadkach 7, 370 05 Ceske Budejovice, Czech Republic, e-mail: tajov@upb.cas.cz*

Millipedes (Diplopoda) are treated as important members of soil macrofauna due to their main role in biodegradation and fragmentation of dead plant material and other soil organic matter. The digestive system of soil invertebrates such as millipedes, takes part in animal body detoxification due to its ability to accumulate toxic substances originated from food or external environment (Nogarol and Fontanetti, 2011). Therefore, the digestive system, especially its endodermal part called midgut, is a good model for studies of external factors effects on the entire organism. However, as the first step of experimental analysis, the knowledge of exact structure and ultrastructure of the midgut wall not exposed to any factors is needed.

The digestive system of *Telodeinopus aoutii* is composed of three distinct regions: the foregut, midgut, and hindgut, which are separated each other by two valves: the cardiac valve, between the foregut and midgut, and the pyloric valve, between the midgut and hindgut. The midgut wall is lined with a columnar epithelium in which all cells have contact with the basal lamina. It is separated from the body cavity by the visceral muscles, which form two layers: an inner layer of circular muscles, and an external layer of longitudinal muscles. Three types of cells can be distinguished among the epithelial cells: digestive cells, secretory cells and regenerative cells. The midgut is accompanied and surrounded by a layer of cells called as "liver", "hepatic cells" or "hepatic tissue". The ultrastructure of *T. aoutii* midgut epithelium has been analyzed using the transmission electron microscopy.

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Cryopreservation of embryogenic tissues: A review

Dariusz Kulus, Małgorzata Zalewska

University of Technology and Life Sciences in Bydgoszcz, Department of Ornamental Plants and Vegetable Crops – Laboratory of Biotechnology, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: dkulus@gmail.com

Cryopreservation (i.e. storage at the temperature of liquid nitrogen; LN – 196°C) is the most safe and cost-effective long-term conservation method of non-orthodox seed species. Somatic embryogenesis, on the other hand, is considered to be the most efficient (micro)propagation technique. By combining *in vitro* tissue culture techniques with cryoconservation it is possible to develop highly-diverse gene banks of both vegetatively and generatively propagated species on a small surface at reduced costs. The application of embryonic tissue for storage in LN is very beneficial, especially with endangered species, since it does not require injuring the mother plant (Lema-Rumińska and Kulus, 2012). The seeds are very often stored at sub-zero temperatures. Their great advantage is the fact that they show a decrease in water content in comparison to vegetative tissues, which is the bottleneck for cryopreservation success. Overtime zygotic embryos or their axes of about 100 species, and somatic embryos of approximately 40 species of plants from different climates have been cryopreserved with variable survival and/or regrowth rates (Engelmann, 2011). The cryopreservation procedures are developed better for the latter ones. For several species, an attempt of freezing embryogenic callus has been also made. This may be a good method for maintaining its embryonic potential. There are even some reports referring to embryogenic potential or metabolic activity growth of proembryogenic masses (PEMs) of some species observed after freezing. In the past various cryopreservation techniques have been applied. As for seeds direct immersion in liquid nitrogen, or simple air-drying (for 1–5 h) is possible. With some species these techniques can be even applied with embryos. Still, the so-called modern methods (e.g. preculture, vitrification, droplet-vitrification or their combinations) are usually more efficient. As for PEMs, the encapsulation-based techniques are the most often applied. There are also reports on employing slow-freezing for embryogenic tissues. All the protocols, however, need to be adjusted not only to the individual needs of species but also even to single cultivars.

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Calreticulin expression in relation to exchangeable Ca^{2+} level that changes dynamically during anthesis, progamic phase, and double fertilization in *Petunia*

Robert Lenartowski[1], Anna Suwińska[2], Marta Lenartowska[2]

[1]*Nicolaus Copernicus University, Faculty of Biology and Environment Protection, Laboratory of Isotope and Instrumental Analysis, Lwowska 1, 87-100 Toruń, Poland, e-mail: rlenart@umk.pl*
[2]*Laboratory of Developmental Biology, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland, e-mail: anmich@doktorant.umk.pl, mlenart@umk.pl*

Calcium (Ca^{2+}) plays essential roles during the progamic phase, gamete fusion, and activation of the fertilized egg in plant sexual reproduction. 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. Our previous studies showed an enhanced expression of *Petunia hybrida CRT* gene (*PhCRT*) during pistil transmitting tract maturation, pollen germination and tube outgrowth on the stigma, and at fertilization and early embryogenesis (Lenartowski et al., 2014). Here, we demonstrate that elevated expression of CRT results in the accumulation of this protein in response to anthesis, pollination, and the late progamic phase and fertilization, when the level of exchangeable Ca^{2+}changes dynamically. On the basis of our results, we discuss the possible functions of CRT with respect to the critical role of Ca^{2+} homeostasis during the key events of the multistep process of generative reproduction in angiosperms.

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Early stages of trunk muscles development in a grass snake (*Natrix natrix*) L. (Reptilia, Colubridae)

Damian Lewandowski[1], Ewelina Posyniak[1], Magda Dubińska-Magiera[1], Weronika Rupik[2], Małgorzata Daczewska[1]

[1]*Departament of Animal Development Biology, Institute of Experimental Biology, University of Wrocław, Sienkiewicza 21, 53-335 Wrocław, Poland, e-mail: malgorzata.daczewska@uni.wroc.pl*
[2]*Departament of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland*

The somites in the grass snake (*Natrix natrix*) as in other vertebrate species, differentiate from unsegmented paraxial mesoderm in a rostral-caudal developmental gradient at early developmental stages. The somites are epithelial structures forming vesicles with centrally located somitocoel. In each somite the outer epithelium forms the cortex. Cortical cells express one of the paired box transcripton factor – Pax3 protein (a marker of muscle progenitor cells). At later developmental stage – 3rd day after egg laying (3dal) the ventral part of the cortex epithelium disaggregates into a mesenchymatic sclerotome, while the dorsal part forms dermomyotome. The dermomyotome in the grass snake is composed of mononucleated cells building structures similar to the dorsomedial and ventromedial lips of the dermomyotome described in birds. The cells of the dermomyotome express Pax3 protein. In successive developmental stage (7 dal), the myotome was composed of mononucleated myotubes. Ultrastructural analysis showed that the sarcoplasm of myotubes were rich in mitochondria, RER, glycogen granules and myofibrils that did not show the regular arrangement characteristic of the mature contractile apparatus. Among the multinucleated myotubes some mononucleated cells were present. Ultrastructural analysis showed that mononucleated cells accompanying differentiating myotubes contained centrally located spindle-shaped heterochromatic nuclei. The nuclei of mononucleated cells were surrounded by a narrow rim of cytoplasm that contained a few RER compartments. Immunocytochemical detection of phosphotylated histon 3 (H3) (a marker of S phase of cell cycle) revealed proliferative activity of these cells. During subsequent stages of myogenesis the dermomyotome was no longer observed. The majority of the myotome was occupied by fast muscles with well developed contractile apparatus. In studied stages of myogenesis, the myotomal muscles were divided into parts by horizontal septa (10 dal). Our preliminary studies of the grass snake myogenesis have revealed that during that process the dermomyotome is a source of progenitor muscle cells which participate in muscle growth (hypertrophy and hyperplasy).

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 grass snake *Natrix natrix* L. is not included in Washington Convention of 1973, ratified by Poland in 1991 (Dz.U. nr 27 poz.112).

Cadmium effect on the coelomocytes in the earthworm *Dendrobaena veneta* (Annelida, Clitellata) coelomic cavity

Anna Majchrzyk, Łukasz Chajec

Department of Animal Histology and Embryology, Silesian University, Bankowa 9, 40-007 Katowice, Poland, e-mail: annamajchrzyk@op.pl, lukasz.chajec@us.edu.pl

Cadmium penetrates from soil into earthworm's body through epidermis and during nutrition with soil. The data of the literature showed that 1,6 – 6% Cd is acumulated in the coelomic fluid and coelomocytes.

The aim of this study was to determine whether Cd present in soil (1) may induce structural and functional changes in coelomocytes floating in coelomic fluid filling up the coelomic cavity of the earthworm *Dendrobaena veneta* and (2) whether the changes were time- and dose dependent.

The ultrastructure of coelomocytes were tested in *D. veneta* earthworms exposed to cadmium at concentrations 10 and 50 mg Cd · kg^{-1} of soil for 10 and 20 days and in the controls.

Electron microscopy studies didn't reveal big and essential changes in ultrastructure of those cells in worms exposed to cadmium for different dose and time.

In earthworms living in cadmium contaminated soil three main cell types of coelomoctes were distinguished in the coelomic cavity: amoebocytes, granulocytes and eleocytes. Eleocytes are big cells containing characteristic large polymorphic granules called chloragosomes, which are surrounded by single membrane. Granulocytes, contrary to the amoebocytes are cells containing polymorphic, electron dense granules. The changes caused by cadmium apply the structure of mitochondria in amoeboctes and granulocytes. In the mitochondria of eleocytes changes were not observed. In comparision to the controls after cadmium intoxication granulocytes and amoebocytes often form important aggregations.

Impact of cultivation duration in liquid medium on regenerative capacity of *Narcissus* L. embryogenic callus

Małgorzata Malik, Karolina Rypel

Department of Ornamental Plants, University of Agriculture in Krakow, Al. 29 Listopada 54, 31-425 Cracow, Poland, e-mail: romalik@cyf-kr.edu.pl

The use of liquid media for multiplication of narcissus embryogenic callus may improve efficiency of the somatic embryogenesis in this species (Malik, 2008; Malik and Molenda, 2008; Malik et al., 2009). The aim of the presented study was to investigate the effect of cultivation duration in liquid medium and inoculum density on biomass growth and somatic embryos formation in embryogenic callus 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-ml Erlenmeyer flasks with 40 ml of medium containing 25 μM Picloram and 5 μM BA for 0 (control) to 120 days and transferred at 10-day intervals on regeneration medium with 5 μM BA and 0.5 μM NAA. The inoculum density was 1:20 and 2:20. The highest intensity of biomass growth in cultures of 2:20 inoculum density between 100 and 110 day of culture were observed. High-density culture multiplicated for a long period of time in liquid medium, proliferate poorly when transferred to solid regeneration medium. In turn, in the lower density cultures better mass growth was noted when they were treated with a liquid medium for longer. Longer duration of propagation in a liquid medium affects the increase in the number of somatic embryos developed on the solid regeneration medium. The optimum cultivation duration in liquid medium is 50 days.

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Effect of ethylene and salicylic acid on somatic embryos maturation of tulip 'Apeldoorn'

Małgorzata Maślanka, Anna Bach

Department of Ornamental Plants, University of Agriculture in Cracow, Al. 29 Listopada 54, 31-425, Poland, e-mail: m.maslanka@ogr.ur.krakow.pl

The presented study was focused on growth and development of tulip somatic embryos. The somatic embryos, at the torpedo stage, were placed for 1 week on media containing growth regulators: (5 mM Picloram and 1 mM 6-benzylaminopurine (BAP) – control), 25 mM Etephon and 25 mM Etephon + 10 mM salicylic acid (SA) in the dark. Then, all the embryos were precultured on media with 2.5 mM BA and 0.25 mM 1-naphthaleneacetic acid (NAA) and maintained in the dark or under light for 10 weeks. Ethylene can stimulate some morphogenetic responses (Maślanka and Bach, 2010) and SA is an inhibitor of its biosynthesis, which also plays an important role in regulating physiological processes in plants (Hara et al., 2012).

The obtained results have revealed that ethylene (released during Etephon breakdown) significantly (nearly two-times) reduced increase in weight and in length of the embryos, in comparison with the control. SA abolished the inhibitory impact of ethylene on the morphology of the embryos, which were similar to the control embryos. After preculture on the medium without Etephon and SA, the differences in the weight and the length were irrelevant. Comparing the light conditions, it was stated, that light stimulates the growth and deformation of the embryos. Previous SA treatment had a positive impact on development of shoots.

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Neuropeptides and peptide hormones influence male reproductive processes in *Tenebrio molitor* beetles

Paweł Marciniak, Arkadiusz Urbański, Monika Szymczak, Milena Kudlewska, Grzegorz Rosiński

Department of Animal Physiology and Development, Adam Mickiewicz University in Poznań, Umultowska 89, 60-614 Poznań, Poland, e-mail: pmarcin@amu.edu.pl, arur@amu.edu.pl, monikasz@amu.edu.pl, rosin@amu.edu.pl

Neuropeptides and peptide hormones are important messenger molecules which influence various crucial physiological processes in insect. They regulate moulting, growth, metabolism, as well as reproduction. While the hormonal regulation of female reproductive processes in insects is extensively studied, still little is known on the regulation of reproductive physiology in males.

Here we report the influence of three peptides Led-NPF-I, *Neb*-TMOF and *Neb*-colloostatin on male reproductive processes in *Tenebrio molitor* beetle. We showed that Led-NPF-I and Neb-TMOF stimulated the endogenic frequency contractions of isolated ejaculatory duct. The effects were reversible and dose-dependent. *Neb*-colloostatin did not change the contractions frequency. The injection of tested peptides in concentration 10^{-5} M into 7-day old males significantly affected the protein content of isolated testes and changed the sperm production. The protein profiles prepared from testes 24h after peptide injection showed changes in expression of proteins with molecular weights ranging from 40 to 100 kDa. Led-NPF-I and *Neb*-TMOF in concentration 10^{-5} M when injected to 7-day old males increased the number of sperm in the testes whereas *Neb*-colloostatin did not change the sperm number. This the first report of hormonal treatment in male beetles that affect reproduction.

rDNA amplification in nurse cells nuclei in polytrophic ovaries of lepidopterans

Marta Mazurkiewicz-Kania

Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Sienkiewicza 21, 53-335 Wroclaw, Poland, e-mail: marta.kania@uni.wroc.pl

Insect ovaries are formed by ovarian tubes termed ovarioles. Traditionally, two main types of ovaries can be distinguished, namely panoistic (follicular oogenesis) and meroistic (nutrimental oogenesis). The oocytes of meroistic ovaries are characterized by a very specific morphology and nuclear metabolism. It results from the presence of nutritive cells (trophocytes) which synthesize a large amounts of rRNA and support the oocyte growth. Besides rRNA (ribosomes), the material transported to the ooplasm (oocyte cytoplasm) also includes certain essential cytoplasmic components such as: maternal RNA (mRNA), mitochondria, lipid droplets and, less frequently, yolk spheres, cortical granules and pigment granules. Therefore, the oocyte nucleus has been transcriptional silenced, however in some studied insect species with meroistic ovary the transcriptional activity of the germinal vesicle has been reported. This activity of oocyte nucleus is morphologically manifested by the present of extrachromosomal rDNA body. Extrachromosomal amplification of rDNA is one of the mechanisms responsible for the multiplication of nuclear organizers. rDNA amplification has been described in the oocytes of several species of different orders of insects. So far massive production of rRNA (ribosomes) performed by selective replication (amplification) of nuclear ribosomal genes (rDNA) in polyploid nurse cells in polytrophic ovaries was described only in Dermaptera. In this report results of histological, histochemical and ultrastructural analyses of germline cells of *Melitaea athalia* (Nymphalidae); *Panthea coenobita, Mythimna conigera* (Noctuidae); *Sphinx pinastri, Laothoe populi* (Sphingidae) are presented. The data obtained indicate that extrachromosomal rDNA amplification can be found in trophocytes of Lepidoptera. Moreover, transcriptional activity of nurse cells nuclei is observed from early previtellogenesis until the advanced stages of oogenesis (vitellogenesis).

Biometric changes caused by the influence of nitrate on the early development of bony fish on example of Labeotropheus trewavasae

Michał Mięsikowski, Barbara Wojciechowska, Natalia Wojciechowska, Bogdana Wilczyńska

Laboratory of Histology and Embryology of Vertebrates, Faculty of Biology and Environmental Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland, e-mail: m.miesikowski@gmail.com, baaasia3@yahoo.com, natalia.w1989@op.pl, wilczyn@umk.pl

Nitrates are an ubiquitous type of substances found in the natural environment. If there is an excessive concentration of those compounds in water, then euthrophic processes are very likely to occur which can result in a further decreased wellness of fish and even cause its death. As far as aquaristics and aquaculture are concerned, nitrates are a potential danger for the biocoenosis; regardless if a water reservoir is professional or just amateurish. The presented experiment aimed to check the dangerous factors caused by nitrates accumulation affecting roe and juvenile forms of some exotic fish in an aquaculture.

The experiment consisted of the observations of the development of Labeotropheus trewavasae under various conditions of the nitrates concentration (0–50; 100; 200; 300 and 400 mg/L). Roe had been taken from a female 24 hours after spawning and later it was placed to an incubator that keeps a precise nitrate concentration (other parameters such as pH, temperature and oxygen concentration were stable in every sample). Six samples has been carried out and each sample included six eggs. The death rate has been ascertained by placing the whole litter to an incubator with a precise nitrate concentration (the examination was repeated 10 times for every single concentration sample). The biomethric researches included: (1) embryonic stage – longitudo ovum, latitudo ovum, longitudo super vitellus, latitudo super vitellus and diameter oculi, longitudo corporis; (2) larval stage – longitudo praedorsale, logitudo captis lateralis, diameter oculi, longitudo praenalis, longitudo corporis, longitudo totalis, altitudo corporis maxima and longitudo et latitudo vesica vitellus. The measurements have been done up to every 48 hours.

The preliminary results show that survival rate decreases along with the nitrate concentration increase and some of the analyzed parameters were actually variable under different content of these chemicals. Possibly, the reason for those correlations will be unraveled in the second part of the experiment which includes the histological researches.

Reproductive properties of *Capsicum* spp. androdiploids derived from *in vitro* anther culture

Lubosława Nowaczyk, Paweł Nowaczyk

University of Technology and Life Sciences, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: nowaczyk@utp.edu.pl

As the result of *in vitro* anther culture of F_1 *Capsicum* spp. hybrids a lot of diploid plants have been obtained. Marker gene analysis showed phenotypic differences between each of them and the donor plant, thus the androgenic origin of diploids has been proofed. Cytometric examination of plantlets suggests that the regenerants developed as the result of spontaneous genome doubling in the early phase of embryogenesis. The presence of peaks 1C, 2C and 4C indicates on the proembryo diploidization while the lack of peak of 1C DNA content in the histograms showed on microspore diploidization. Independently of presented possibilities of genome doubling this way of diploid creation gives homozygous plants without the colchicine treatment. The usage of the mentioned mutagenesis agent produce the significant number of mixoploid plants. Moreover a number of treated plants decay in the result of colchicine activity. From this point of view the spontaneous diploids (androdiploids) appeared in the anther culture are very interesting material for molecular genetic analysis as well as for plants genetic improvement. In the evaluation of reproductive properties of androdiploids the attention was turned to the placenta and seeds weight, seeds weight and number. These features are very informative about the plant fertility. Then the high fertility indicates on genetic stability of plant, expressing by the lack of the problems during the gamete- and embryogenesis. I the presented experiment four donor F_1 hybrids and four androdiploid populations were used. In each of the case the volume of examined feature of androdiploids was lower than in the related donor plant and close to the female partner of F_1 hybrid.

Apomictic progeny of *Capsicum annuum* L. androgenic haploid – phenotypic and molecular analysis

Lubosława Nowaczyk[1], Paweł Nowaczyk[1], Dorota Olszewska[2], Aleksandra Niklas-Nowak[2]

[1]*University of Technology and Life Sciences, Bernardyńska 6, 85-029 Bydgoszcz, Poland, e-mail: nowaczyk@utp.edu.pl*
[2]*University of Technology and Life Sciences, Kaliskiego 7, 85-789 Bydgoszcz, Poland, e-mail: dorota@utp.edu.pl*

The plants of doubled haploid line, marked as 15, derived from hybrid of `Corno di toro` cultivar and ATZ breeding line were the initial material of investigation. The DH line was similar to the maternal form of the hybrid in relation to plant and fruits habit. Among the haploids, obtained as result of *in vitro* anther culture, the plants with not numerous, very small fruits have been observed. One of these plants (denoted 15/1x), which set 10 fruits, was under evaluation. During fruit analysis eight well developed seeds was found. Inside the fruits there was not the placenta observed. The seeds have been set on septum tissue. For comparison, the number of seeds from diploid plant (denoted 15/2x), derived also from the same anther culture, reached 2.568. In our opinion the diploid embryos developing on haploid plant are apomictic in their originIn the next year, eight seeds collected from fruits of haploid gave normally developed diploids. These plants, as well as the progeny of 15/2x were the material for morphological analysis. The standard deviation of features showed bigger differentiation in offspring of haploid plant and the attention was paid to three of plants with significantly low weight of the fruit. All of the plants were the subject of RAPD molecular analysis and ten primers (A06, A10, A11, A14, A17, A19, OPB10, OPAE10, OPAE11, OPAE19) were used. The size of amplified products ranged from 360–952 bp. Three plants mentioned above were original with regard to three of the primers: presence of amplified products – 554bp (OPAE11), and lack of amplification – 435bp (OPAE10) and 788bp (OPB10). The results of analysis suggest that the primers used were in the relation with the gen/s responsible for fruit weight, which is conditioned by fruit shape, length, width and thickness of pericarp.

Endogenous hormone concentrations in *Arabidopsis* explants undergoing induction of somatic embryogenesis

Katarzyna Nowak, Barbara Wójcikowska, Agnieszka Kiwior-Wesołowska, Anna Wójcik, Daria Grzybkowska, Małgorzata Gaj

Department of Genetics, Faculty of Biology and Environment Protection, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland, e-mail: kmanka@us.edu.pl, barbaragzyl@gazeta.pl, agnieszka.kiwior-wesolowska@us.edu.pl, ania.karwot@gmail.com, dariagrzybkowska@interia.pl, malgorzata.gaj@us.edu.pl

Somatic embryogenesis (SE) results in embryo development from somatic cells in response to inductive *in vitro* conditions. SE is widely used in biotechnology in micropropagation and genetic transformation. Studies on SE provide a useful system to reveal the molecular mechanisms of zygotic embryogenesis and *Arabidopsis*, a model in plant genomics, is recommended to identify genetic regulation involved in embryogenic transition of somatic cells.

The interplay between transcription factors (TFs) and phytohormones in control of differentiation of plant cells is widely accepted. Accordingly, in SE of *Arabidopsis* the intensive modulation of hormone-related TFs was indicated implying changes of phytohormone content in embryogenic culture (Gliwicka et al., 2013). Hence, the aim of this work was to quantify the endogenous concentrations of different phytohormones at different time points of *Arabidopsis* SE culture, including 0d, 3d, 5d, 10d and 15d. Hormones were quantify by the HPLC method and the analysis involved determination of auxin (IAA), abcisic acid (ABA), jasmonic acid (JA), salicic acid (SA) and 8 different types of cytokinins (trans-zeatin, trans-zeatin-rybosid, dihydrozeatin, dihydrozeatin-rybosid, isopentenyl-adenine, trans-zeatin-O-glucosid, trans-zeatin-O-glucosid-rybosid, cis-zeatin). The analysis showed a significantly modulated concentration of most of the studied hormones during SE. However, the concentration and the profiles of the observed changes differed between hormones. A content of IAA, a hormone believed to be a key in SE induction, was found highly increased in SE supporting the involvement of auxin biosynthesis in mechanisms operating during SE induction. In contrast to IAA, the concentration of stress hormones, ABA and JA, were significantly decreased during a progress of SE suggesting their negative control in embryogenic induction. In contrast to ABA and JA, a high level of SA in explants undergoing induction of embryogenic was noticed. In cytokinins, the concentrations and profile of their changes were found highly dependent on the type of the hormone.

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A description of the early life history stages of *Cheilochromis euchilus* (Trewavas, 1935) (Pisces, Cichlidae) from Lake Malawi under laboratory condition

Roman Pawlak[1], Katarzyna Wołczuk[2]

[1]*Museum of Natural History, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: pawlak@umk.pl*
[2]*Laboratory of Histology and Embryology of Vertebrates, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: katekoz@wp.pl*

The early ontogeny of endemic to Lake Malawi cichlid fish *Cheilochromis euchilus* was described from egg activation to total yolk sac resorption. For development, we defined three periods: the embryonic, larval and juvenile. For embryonic development – 4 dpf (day post fertilization), defined 7 stages, which were named: zygote, cleavage, blastula, gastrula, segmentation, pharyngula (heart beat) and hatching. For larval development -15 dpf, defined three stages, which were named: protolarva (p), mezolarva (m) and metalarva. For juvenile development until 19 dpf, defined one stage in the early juvenile period (j).

In the embryonic period, the following features were measured: eggs length – 4,25±0,05 mm, eggs width – 3,01±0,29 mm, perivitelline length 0,29±0,13 mm. During the larva and juvenile stages the following parameters were measured: total body length (p – 5,98±0,05 mm, m – 8,78±0,06 mm, j – 14,58±0,06 mm); head height (p – 0,80 mm, m – 1,38 mm, j – 2,82 mm), head length p – 1,85 mm, m – 2,23 mm, j – 3,45 mm, diameter of eye (p – 0,66 mm, m – 0,89 mm, j – 1,02 mm); length (p – 3,06 mm, m – 2,78 mm) and height (p – 3,14 mm, m – 2,84 mm) of the yolk sac.

Clitellate phylogeny based on the female reproductive system and other morphological characters

Dorota Pikuła[1], Joanna Maria Cichocka[1], Aleksander Bielecki[1], Piotr Świątek[2], Anna Z. Urbisz[2], Iwona Jeleń[1], Bartosz Płachno[3]

[1]*Department of Zoology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-719 Olsztyn, Poland, e-mail: dorota.pikula@uwm.edu.pl, joanna.cichocka@uwm.edu.pl, alekb@uwm.edu.pl, iwona.jelen@uwm.edu.pl*

[2]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl, anna.urbisz@us.edu.pl*

[3]*Department of Plant Cytology and Embryology, Jagiellonian University in Cracow, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: bartosz.plachno@uj.edu.pl*

The study demonstrates hypothetical phylogeny of Clitellata based on 31 characters of the ovary organization and course of oogenesis, as well as the male reproductive system and general morphology. Analyses included 14 representatives of 10 families belonging to Lumbriculida, Haplotaxida, Branchiobdellida, Acanthobdellida and Hirudinida. Phylogenetic reconstruction was performed with PAUP* (Swofford, 2000) under the branch-and-bound option, and statistical support for clades was assessed using bootstrap analysis and TBR branch swapping. Parsimony analysis resulted in 102 equally most parsimonious trees (length = 30, CI = 0.90, RI = 0.96). The strict consensus of these trees resolved 5 clades out of a total 13 possible. Highly supported monophyletic clades include traditional Euhirudinea considered in present study as *Branchiobdella* + *Acanthobdella* + Hirudinida, and the family Hirudinidae. *Acanthobdella peledina* appeared to be in closer relation to Hirudinida than *Branchiobdella parasita*. Traditional groups of rhynchobdellid and arhynchobdellid leeches were not confirmed. Other clitellate taxa formed separate evolutionary line each. The features used in our analysis appeared to be highly conservative, but they allow to infer about the phylogeny of Clitellata in terms of traditional division in Oligochaeta and Hirudinea. The study is the first attempt to use the ultrastructural female reproductive features in a set of characters for phylogenetic analysis and in the future the inference should be expended by another phylogenetic informative data like it is in the papers Marotta et al. (2008) and Bielecki et al. (2013).

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Intercellular bridges in the oogenesis of Parachela (Tardigrada, Eutardigrada)

Izabela Poprawa, Marta Hyra, Michalina Kszuk-Jendrysik, Lidia Sonakowska, Magdalena Rost-Roszkowska

Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: izabela.poprawa@us.edu.pl, martah1988@o2.pl

During oogenesis in tardigrades, which belong to the order Parachela, germ cells undergo incomplete cytokinesis to form clusters of cystocytes interconnected by stable intercellular bridges (cytoplasmic bridges). Among cells forming the cluster only one cell develops into the oocyte, while the remaining cells become the nurse cells (trophocytes) (Poprawa, 2005). Stable intercellular bridges allow the directional transport of nutrients and organelles between interconnected cells. Ultrastructural analysis has shown that the cytoplasmic bridges in female germline of Parachela form the standard ring canals. Two types of these connection have been observed. The first type has been observed in *Isohypsibius granulifer granulifer* and *Xerobiotus pseudohufelandi*. In these cases the ring canal consists of two rims: the outer rim is electron dense, while the inner rim has less electron density. Similar ultrastructure of intercellular bridges has been decsribed during the oogenesis in *Drosophila melanogaster*. The second type of cytoplasmic bridges observed in female germline of Parachela is similar to that described in spermatogenesis of *D. melanogaster* (Haglund et al., 2011). In these cases the ring canal consists mainly of one electron dense rim. The second type of intercellular bridges has occurred in the germline of *Dactylobiotus dispar*, *Dactylobiotus parthenogeneticus* and *Macrobiotus polonicus*. During oogenesis of those species the diameter of a ring canal increases (particularly between trophocytes and oocyte). In *I. g. granulifer* it increases from 1.25 μm during previtellogenesis to 2.3 μm during the late vitellogenesis.

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Somatic embryogenesis of *Cyathea delgadii*: from somatic cell to somatic embryo

Mariusz Pożoga, Anna Mikuła, Jan J. Rybczyński

Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: mariuszpozoga@gmail.com

Cyathea delgadii is a tree fern species for which somatic embryogenesis (SE) process was developed. We introduce non hormonal system for somatic embryo production. Excised 2,5 mm long fragments of stipes, from 3 to 5 frond sporophytes cultured in darkness, placed on half strength Murashige and Skoog medium (1/2 MS) supplemented with 1 % sucrose and solidified with 0,7 % agar produced more than 40 somatic embryos during 2 months. More than 80 % of placed explants induce SE.

Using this non hormonal system of induction of (SE) we documented stages of somatic embryo development. Different kinds of microscopy were used. We tried to define particular stages of somatic embryo development.

SE starts when somatic cell of epidermis divide anticlinal on two equal cells. Next divisions are also anticlinal and its number is variable. After series of anticlinal divisions periclinal divisions occure. In effect of later mixed peri- and anticlinal divisions somatic embryo become structure of four slightly separated parts. Thereafter embryo take globular form and first leaf occurs. When first leaf has visible fiddlehead second leaf starts to develop.

C. delgadii can become in future convenient model for research in field of somatic embryogenesis in *Pteridophyta*.

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The influence of progesterone on *Arabidopsis* female gametophyte cultured *in vitro*

Joanna Rojek, Aleksandra Motyka, Joanna Kałon, Małgorzata Kapusta, Jerzy Bohdanowicz

Department of Plant Cytology and Embryology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, e-mail: joanna.rojek@biol.ug.edu.pl

Mammalian sex hormones (MSH), which include androgens, estrogens and progesterone play a key role in the reproduction in mammals. It has been proven the existence of endogenous progesterone in plant species (Janeczko, 2012). Selected steroids deposited exogenous to plant stimulate cell division, the maturation of pollen as well as growth and flowering. The newest reports suggest that exogenous MSH application may be used to increase the plant resistance to enviromental stress (Janeczko et al., 2013). It is thought that the activity of progesterone in plants will increase, if in vitro method is applied. Our previous research showed that in vitro conditions induce autonomous development of unfertilized central cell in non-mutated genotypes, reminiscent *fis*-class mutants in planta (Rojek et al., 2013). To check whether and how progesterone influence on generative development of *Arabidopsis*, the unfertilized ovules of non-mutated and *fis*-class genotypes were cultured on media with different concentration of progesterone (PR), also combined with auxin and cytokinin. Ovules and female gametophytes inside cultured ovules were characterized by an evident enlargement. On the 5 of 7 media used the autonomous endosperm (AE) induction and development were noted, with the highest frequency on the medium with 1μM PR. The significant delay of AE development we observed in vitro as compare to endosperm after fertilization. Although, the stages just before cellularization of AE we noted in *MEA/mea*. The AE occurrence in all genotypes and advanced stages of AE development in *mea* mutant, in the presence of progesterone newly applied *in vitro*, are the evidences supporting the hypothesis about the ability of *Arabidopsis* species to apomictic-like development.

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The cell death in the midgut and hepatopancreas of the freshwater shrimp *Neocaridina heteropoda* (Crustacea, Malacostraca)

Magdalena Rost-Roszkowska, Lidia Sonakowska, Izabela Poprawa, Karolina Kamińska, Michalina Kszuk-Jendrysik, Marta Hyra, Bartłomiej Zajusz, Agnieszka Włodarczyk

Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: magdalena.rost-roszkowska@us.edu.pl, sonakowskal@gmail.com, izabela.poprawa@us.edu.pl

The alimentary system of *Neocaridina heteropoda* (Crustacea, Malacostraca) is composed of three basic regions: the foregut, the midgut, and the hindgut. The midgut is surrounded by a large, lobed digestive gland or hepatopancreas. The simple epithelium of the midgut is formed by digestive and regenerative cells, while among epithelial cells of hepatopancreas three types of cells have been distinguished.

Three kinds of the cell death have been observed in the midgut and hepatopancreas epithelium: apoptosis, autophagy and necrosis. Autophagy seems to be a common process observed in the cytoplasm of midgut cells in both analyzed organs. At the beginning of autophagy, the cytoplasm and organelles are surrounded with double-membraned phagophore, which gradually enlarges. The closure of phagophore membranes results in the formation of double-membraned structures called autophagosomes. After the fusion of autophagosomes and lysosomes, the autolysosomes are formed. Finally, the organelles enclosed inside the autolysosomes undergo gradual digestion. Together with autophagy, the apoptosis and necrosis have been observed in the cytoplasm of digestive cells of the midgut and epitehlail cells of the hepatopancreas. Autophagy has been described with the use of the light and transmission electron microscopes, and histochemical methods (dansylcadaverine staining, acid phosphatase stining). Apoptosis has been analyzed using light microscope, transmission electron microscope and immunostaining methods (TUNEL assay, acridine orange).

Gonad aging in *Xenopus laevis*

Beata Rozenblut-Kościsty, Magdalena Chmielewska, Maria Ogielska

Department of Evolutionary Biology and Conservation of Vertebrates, University of Wroclaw, Sienkiewicza 21, 50-335 Wroclaw, Poland, e-mail: brozenblut@biol.uni.wroc.pl

Studies on structure and function of gonads in amphibians mainly relate to sexually mature individuals actively involved in reproduction, or sexual differentiation and development of gonads. Only few data are available on gonads of aging amphibians because senile individuals are very rare. Most of publications still refers to the (false) theory that oogenesis in amphibians is cyclic and the oocyte stock is restored every season by proliferation of rudimental oogonia located in germ patches. According to this theory, ovaries should not exhibit aging symptoms, similarly to testes, (Kara, 1994; Kulkarni and Pancharatna, 1996).

Histological analysis of gonads was conducted on 15 years old *Xenopus leavis* (9 females and 5 males). The reference group consisted of 4 ovaries and 4 testes of oneyearold individuals, and 1 testis of sexually mature young male. Gonads were fixed in Bouin, 5–7μm thick paraffin sections were stained with Mallory's trichrome. General morphology of the gonads was analyzed using light microscopy with special respect to frequency and degeneration of specific stages of germ cells, as well as pigment apposition characteristic to senile amphibian tissues.

Tunica albuginea in the testes of senile males was thicker and richer in collagen fibers than of juveniles. Increased amount of collagen fibers was also observed around seminiferous tubules and *rete testis*. Diameters of seminiferous tubules were bigger than in juvenile testes, and the tubules were filled with primary and secondary spermatogonia, spermatocytes and bundles of spermatids. The vast majority of spermatids had an abnormal structure. Degeneration was observed at all stages of germ cells. *Theca externa* in senile ovaries was thickened and rich in pigment aggregations, which was absent in gonads of juvenile and sexually active females, and vitellogenic oocytes predominated over previtellogenic ones. Germ patches were extremely rare. Senile ovaries and testes displayed the intense penetration of blood vessels into the center of gonads, which was absent in juvenile gonads. Noteworthy is the presence of histologically abnormal tissue containing gonial germ cells in one testis and one ovary.

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Morphological studies of the sand lizard *Lacerta agilis* L.* (Reptilia, Lacertidae) embryos

Weronika Rupik[1], Elwira Swadźba[1], Magdalena Kowalska[1], Hanna Jackowiak[2], Robert Maślak[3]

[1]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: weronika.rupik@us.edu.pl, elwira.swadzba@us.edu.pl, magda222360@wp.pl*
[2]*Department of Histology and Embryology, Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland, e-mail: hannaj@up.poznan.pl*
[3]*Department of Vertebrate Biology and Conservation, University of Wrocław, Sienkiewicza 21, 50-225, Wrocław, Poland, e-mail: maslak@biol.uni.wroc.pl*

For oviparous animals such as many reptilian species, oviposition marks the transition between embryonic development within the mother and in the environment. The stage of development at oviposition differs among reptilian groups. For example, for the great majority of squamates, development proceeds normally while eggs are in utero, and by the time of oviposition, embryos are approximately one-third through development (Andrews and Mathies, 2000). The purpose of this study was to examine external morphological development between oviposition and hatching of the sand lizard embryos. The sand lizard is most common lizard species in Poland and Europe and west Asia. 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 material was fixed in 10% formalin solution and maintained in 1:1 mixture of absolute alcohol and glycerol. The model collection included 60 embryos isolated during each incubation day from hatching. Morphological description was based on the analysis of embryonic developmental characters examined under stereo microscope. The list of diagnostic characters was similar as were used for morphological description of grass snake embryos (Rupik, 2002). This morphological study will be the base for the construction of the developmental table.

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 shape of fetal ossification centers may indicate malformation of vertebral column

Agnieszka Skórzewska, Małgorzata Grzymisławska, Małgorzata Bruska, Joanna Łupicka, Witold Woźniak

Department of Anatomy, University of Medical Sciences, Święcickiego 6, 60-781 Poznań, Poland, e-mail: a.skorzewska@gmail.com

The vertebral clefts being the variations of the endochondral ossification if coexists with additional abnormalities of skeletal system or internal organs may indicate malformations of the vertebral column (Tanaka and Unthoff, 1983; Westvik and Lechman, 1996).

The number and shape of ossification centers were evaluated in nine fetuses aged 11–21 weeks in computed tomography study.

It was observed that ossification of vertebrae commences at the end of 10th week. In neural arches it appears first in the cervical and upper thoracic vertebrae and proceeds in craniocaudal direction. In vertebral centra ossification was observed in the lower thoracic and first lumbar vertebrae in fetus aged 10 weeks and in two lower cervical, all thoracic and lumbar, and three upper sacral vertebrae at age 11 weeks. Ossification centers for C5 center were visible at age 12 weeks and at age 13 weeks in vertebral centra C2 to C4. Particular attention was paid to the shape of ossification centers for vertebral centra. In all investigated fetuses they were round or oval in cervical and sacral vertebrae but varied in shape in thoracic and lumbar vertebrae. The mushroom-shaped, C-shaped or irregular ossification centers were observed as well as consisting of two parts connected by thin bony bridges and resembling the shape of letter H or horizontally oriented letter U.

In fetus aged 18 weeks two parts of ossification centers for thoracic and lumbar vertebral centra were completely separated. The fetus did not demonstrate any other abnormalities.

Observed coronal vertebral cleft is considered as a variation of normal endochondral ossification and usually disappears until the second year of life. Coronal cleft appearing in rare congenital malformations of skeletal system coexists with craniofacial dysmorphia and multiple skeletal anomalies such as shortening of long bones, hemivertebrae, butterfly or hypoplastic vertebrae, supernumerary ribs, polydactyly, and congenital disorders of internal organs.

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Ultrastructural changes of the thyreocytes during shedding complex formation sand lizard *Lacerta agilis* L. (Reptilia, Lacertidae) embryos

Elwira Swadźba[1], Magdalena Kowalska[2], Weronika Rupik[2]

[1]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: elwira.swadzba@us.edu.pl*
[2]*Department of Animal Histology and Embryology, University of Silesia, Bankowa 9, 40-007 Katowice, Poland, e-mail: magda222360@wp.pl, weronika.rupik@us.edu.pl*

The hormonal activity of the thyroid plays important roles during the development and growth of animals. In reptilian species, thyroid activity particularly affects the formation of the shedding complex and regulation of the sloughing cycle (Rupik and Swadźba, 2009; 2010; 2011). The eggs of *Lacerta* were incubated in constant temperature at 30°C and the embryos were isolated in regular sequence of time. The age of embryos was calculated using the table of species development. Throughout developmental stages 17–20, the thyroid primordium contained undifferentiated cellular cords. At developmental stages 21–24, the thyroid anlage was composed of small follicles with lumens. The Golgi complex and the RER developed gradually at developmental stage 25–30. At developmental stage 32, most follicles were outlined by squamous epithelial cells and presented wide lumens filled with a light colloid. The Golgi complex and RER showed changes in their morphology indicating a decrease in the activity of the thyroid gland. At developmental stage 34, the activity of the embryonic thyroid gradually increased, and at the 35th stage, it exhibited the features of a fully active gland. The follicular epithelium cells frequently showed merocrine secretion into follicular lumens. Subsequently activity of thyroid gradually decreased (stages 37–39) and at the time of hatching, it exhibited the features of inactive gland. These ultrastructural changes of thyroid cells coincide with the changes in the differentiating epidermis of sand lizards embryos. The initial shedding complex is formed under the increasing activation of thyreocytes at the developmental stages 34–35. Before hutching the periderm layer detached *in ovo* and this first molting occurred when the thyrocyces were hypoactive.

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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Mitotic and meiotic chromosomes of the Great Rams Horn Snail *Planorbarius corneus* (Linnaeus, 1758) (Gastropoda, Planorbidae) from Lake Kortowskie

Aleksandra Szabelska, Dorota Juchno, Aneta Spóz, Alicja Boroń

Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-718 Olsztyn, Poland, e-mail: aleksandra.szabelska@uwm.edu.pl

Aquatic organisms are exposed to a progressive degradation of their living environment that may lead to changes in their functional morphology, including the level of genomes and chromosomes. One of the effects of the pollution of aquatic environments may be a disturbance in the process of meiosis for living organisms, such as gastropods. The aim of this present study was the analysis of meiotic and mitotic chromosomes of *P. corneus* individuals inhabiting Lake Kortowskie.

An analysis of meiotic and mitotic chromosomes of *P. corneus* inhabiting Lake Kortowskie was made in order to verify the use of different tissues and colchicine treatments, the hypotonization time and two methods of chromosome slide preparation. In total, 30 chromosomal slides of six individuals were analyzed. The well spread chromosomes were introduced onto the slides by dropping a cell suspension of the mantle epithelium, foot and intestine of each individual, directly injected with colchicine, after 20 min of hypotonization. The karyotype was composed of 2n=36 biarmed chromosomes, thirty metacentrics with the rest being submetacentrics, NF=72. In the gonad, the meiotic chromosomes in spermatogenesis were observed as being in prophase I (leptoten, zygoten, and diakinesis) and in telophase I. In diakinesis 18 bivalents were formed. No disturbances were observed during meiosis. The spermatozoa were typical of aquatic molluscs; consisting of a spherical head, a short midpiece and a long tail.

The results presented here do not reveal any differences between the karyotype of the Great Rams Horn snail from Lake Kortowskie and the karyotypes formerly reported in the published literature, but only confirmed data on the karyotype of this species. However, the results contributed new data on meiotic chromosomes, and the spermatozoa of this species. Insightful observation of meiosis may in the long-run perspective allow the recording of disturbances in this process among snails, caused by water pollution.

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Ovary structure of *Matsucoccus pini* (Insecta, Hemiptera, Coccinea: Matsucoccidae)

Teresa Szklarzewicz[1], Anna Michalik[1], Małgorzata Kalandyk-Kołodziejczyk[2], Michał Kobiałka[1], Ewa Simon[2]

[1]*Department of Developmental Biology and Morphology of Invertebrates, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: teresa.szklarzewicz@uj.edu.pl*
[2]*Department of Zoology, Faculty of Biology and Environmental Protection, University of Silesia, Bankowa 9, 40-007 Katowice, Poland*

The ovaries of *Matsucoccus pini* are composed of about 50 ovarioles of telotrophic type that develop asynchronously. The ovarioles are radially arranged along two thirds of the length of the lateral oviduct. The ovarioles consist of three well defuned regions: the tropharium (trophic chamber), vitellarium, ovariolar stalk (pedicel). The terminal filament is absent. The analysis of serial semithin sections in 10 ovarioles has revealed that ovarioles enclose 32 interconnected germ cells: from 23 to 27 trophocytes and from 5 to 9 oocytes. The tropharia are long, tubular and contain individual trophocytes (nurse cells) and early previtellogenic oocytes (arrested oocytes) that are capable of further development. In the centre of the tropharium a common cytoplasmic area termed a trophic core occurs. The trophic core is devoid of cells and is connected both with trophocytes and oocytes. Trophocytes communicate with the trophic core by cytoplasmic processes, oocytes by nutritive cords. In contrast to other scale insects, vitellaria of *Matsucoccus pini* comprise several oocytes. The developing oocytes are surrounded by a single layer of follicular cells that do not diversify into subpopulations. In the early vitellogenic oocytes the accessory nuclei arise. In the cytoplasm of trophocytes, arrested oocytes and developing oocytes numerous rod-shaped microorganisms are present. The obtained results indicate that ovarioles of *Matsucoccus pini* represent the same structure as those of *Steingelia gorodetskia* (Steingeliidae) (Koteja et al., 2003). The plesiomorphic characters of ovarioles of *M. pini* and *S. gorodetskia* (i.e. tubular shape of the tropharium, large number of germ cells constituting ovarioles, presence of arrested oocytes capable of further development, occurrence of several oocytes in the vitellarium) indicate the basal position of Matsucoccidae and Steingeliidae.

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Early development of the skeleton of cobitid fishes (Teleostei, Cobitidae)

Jolanta Szlachciak[1], Dorota Juchno[1], Alicja Boroń[1], Roman Kujawa[2]

[1]*Department of Zoology, University of Warmia and Mazury, Oczapowskiego 5, 10-719 Olsztyn, Poland, e-mail: jolasz@uwm.edu.pl, juchno@uwm.edu.pl, alibo@uwm.edu.pl*
[2]*Department of Lake and River Fisheries, University of Warmia and Mazury, Oczapowskiego 5, 10-719 Olsztyn, Poland, e-mail: reofish@uwm.edu.pl*

The aim of the present study was to provide a description of the early development of the skeleton in karyologically identified *Cobitis taenia* Linnaeus, 1758 (Cobitidae). Males and females were caught from an exclusively diploid population (Lake Legińskie, Poland) in June of 2009 and 2010. Controlled reproduction took place at the hatchery of the Department of Lake and River Fisheries. The collected specimens after fixing in formalin were transferred to the 70% alcohol, then cleared in trypsin, differentially stained with alcian blue and alizarin red according to modified method of Dingerkus and Uhler (1977). Observations were made one day post hatching using the microscope Olympus CX41 equipped with digital camera.

All specimens examined have well developed cartilaginous elements of the neurocranium: auditory capsules, parachordal plate and trabecular bars. The trabecular bars were joined anteriorly with the ethmoid plate. The notochord was visible as straight rod extending for the entire length of the body. The pectoral fins were visible.

At hatching, larvae can be at various states of development. It mainly depends on the size of the yolk. The order of appearance and development of cartilaginous elements are highly variable in teleosts. In Salmonidae the development of the skull and fins starts well before hatching, while in Sparidae the skull and fins develop after hatching (Koumoundouros et al., 1999). In analyzed specimens of spined loach only some elements of chondrocranium were formed. No skeletal element was observed in an axial skeleton.

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Somatic cells of the ovariuterus of two katoikogenic scorpions *Ophistothalamus boehmei* and *Heterometrus spinifer* (Chelicerata, Scorpiones, Scorpionidae)

Kamil Szymusiak, Izabela Jędrzejowska

Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Sienkiewicza 21, 53-335 Wroclaw, Poland, e-mail: kamil.szymusiak@uni.wroc.pl, izabela.jedrzejowska@uni.wroc.pl

In scorpions due to the fact that both fertilization and embryonic growth are held within scorpion female gonad, the gonad is referred to as *ovariuterus*. The ovariuterus is a ladder-like structure, shaped by longitudinal and transverse tubes, which consists of germline cells surrounded by somatic tissue. In scorpions, like in most chelicerates, growing oocytes protrude outside the wall of the gonad in accompany of somatic cells. The scorpion ovariuteri have been divided into two types: apoikogenic and katoikogenic. Each type exhibits several unique features, concerning the position of growing oocytes, their relations with somatic cells and accumulation of reserve materials. In apoikogenic type oocytes grow in follicles connected to the ovariuterus wall by the oocyte stalks, while in katoikogenic type the oocyte growth proceeds in pockets (*diverticula*) of the ovariuterine wall.

In *Ophistothalmus boehmei* and *Heterometrus spinifer* from family Scorpionidae, the *ovariuterus* is of katoikogenic type. The wall of ovariuterine tube shows a peculiar structure. Namely it consists of two layers of epithelia. With the onset of previtellogenesis the oocytes protrude from ovariuterus to its outpocketings, called *diverticula*, composed of two layers of epithelial cells: outer and inner ones. The outer layer is exposed to mesosomal cavity, whereas the epithelial cells of inner layer either adhere to the oocyte or face to each other. During oocytes growth, *diverticula* elongate, and oocytes resides in terminal parts of *diverticula*. In fully grown *diverticula*, external epithelium layer consists of small, flattened cells. Within internal epithelium three distinct parts can be distinguished: 1) In terminal area of diverticulum, epithelial cells with their apical parts adhere to oocyte surface, whereas their basal parts contact the basement membrane. These cells are referred to as the follicular cells. 2) In mid-part of *diverticulum* the epithelial cells are organized into a simple columnar epithelium. Their apical parts adhere to each other by the means of microvilli, resembling the structure of the oocyte stalk in apoikogenic ovariuterus. 3) In basal parts of *diverticulum*, epithelial cells move apart, extending *diverticula* lumen towards the *ovariuterus*. However, the lumen of the *diverticulum* does not continues to the lumen of the *ovariuterus*. This enclosure seems to prevent premature oocyte fertilization.

Somatic embryogenesis of the tree fern *Cyathea delgadii*: ploidy stability of embryo-derived sporophytes

Karolina Tomiczak[1], Mariusz Pożoga[1], Anna Mikuła[1], Elwira Śliwińska[2], Jan J. Rybczyński[1]

[1]*Department of Experimental Plant Biology, Polish Academy of Sciences Botanical Garden – Center for Biological Diversity Conservation in Powsin, Prawdziwka 2, 02-973 Warsaw, Poland, e-mail: ktomiczak@obpan.pl*
[2]*Department of Plant Genetics, Physiology and Biotechnology, University of Technology and Life Sciences in Bydgoszcz, Prof. S. Kaliskiego 7, 85-789 Bydgoszcz, Poland*

The phenomenon of somatic embryogenesis has been described for tissue cultures of various angio- and gymnospermous plant species as well as for two clubmosses *Lycopodiella inundata* and *Huperzia selago*. The tree fern *Cyathea delgadii* is the first species belonging to the monilophyte clade, for which the process of somatic embryogenesis has recently been recognized. This discovery makes it possible to study the events occurring during induction and development of a fern somatic embryo on the molecular, biochemical and physiological levels, which can improve our understanding of evolutionary aspects of the whole process.

Primary somatic embryos of *C. delgadii* were initially regenerated from gametophyte-derived haploid calli and from diploid zygotic embryos. Secondary somatic embryogenesis can be easily induced on the intact somatic embryos and on the stipes of 2-frond somatic embryo-derived sporophytes cultured on a half-strength Murashige and Skoog basal medium without the addition of any plant growth regulators. However, because somatic embryos can be obtained from both haploid and diploid tissues, it is crucial to control the ploidy level of plant material regenerated *in vitro*. The aim of the work was to compare the total nuclear DNA content and the number of chromosomes of *C. delgadii* embryo-derived sporophytes with their source explants. For flow cytometry analysis fronds of young sporophytes were used and *Petunia hybrida* 'P × Pc6' (2.85 pg/2C) served as an internal standard. Metaphase chromosomes were counted in root-tip meristems following the method of the Feulgen staining. The results showed that embryo-derived sporophytes maintained the same nuclear DNA content and chromosome number as the initial plant material, thus they were stable in terms of ploidy.

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Morphology and functioning of germline stem cell niches in the panoistic ovarioles of a basal "apterygotous" insect, *Thermobia domestica*

Wacław Tworzydło, Elżbieta Kisiel, Władysława Jankowska, Szczepan M. Biliński

Department of Developmental Biology and Morphology of Invertebrates, Institute of Zoology, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: szczepan.bilinski@uj.edu.pl

In the model organism, the fruit fly, *Drosophila melanogaster*, the germline stem cells (GSCs), similarly to the germline and non-germline stem cells of other species, function in specialized microenvironments formed by somatic cells, referred to as the niches. In *Drosophila* ovaries, the female GSCs divide asymmetrically to produce new GSCs and the progenitor cells, the cystoblasts. Each cystoblast then divides to generate the cyst composed of 16 interconnected sibling cells, the cystocytes. After cyst formation, specific molecules are transported to one of the cystocytes which differentiates into the oocyte, while the other 15 cells become the nurse cells (trophocytes).

We have studied morphology and functioning of the GSC niches in the panoistic ovarioles (ovaries) of a basal "apterygotous" insect, *Thermobia domestica*. We show that in this species, the GSCs are present along the anterior apex of the germarium. Individual GSCs are separated from each other and from basement lamina covering the ovariole by characteristic somatic cells, termed the apical somatic cells (ASCs) or their elongated processes. We believe that all the ASCs of a given ovariole constitute a "dispersed" niche in which GSCs are anchored. Our analyses have additionally shown that in *Thermobia*, both the cystoblasts and young oocytes are always individual and never form syncytial cysts. These findings indicate that in certain basal insects, the syncytial phase of oogenesis has been eliminated during evolution.

Finally, we show that in the early meiotic oocytes of *Thermobia*, during so-called bouquet stage, prominent Balbiani bodies are formed. the Balbiany bodies always arise next to this segment of the nuclear envelope to which the telomeres of the bouquet chromosomes are attached. In the light of these data, we suggest that the localization of the Balbiany body together with the polar attachment of the bouquet chromosomes play a crucial role in the asymmetrization of *Thermobia* oocytes.

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Allometric growth of the alimentary tract in the Siberian hamster (*Phodopus sungorus*)

Katarzyna Wołczuk[1], Roman Pawlak[2]

[1]*Laboratory of Histology and Embryology of Vertebrates, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: katekoz@wp.pl*

[2]*Museum of Natural History, Faculty of Biology and Environment Protection, Nicolaus Copernicus University, Lwowska 1, 87-100 Torun, Poland, e-mail: pawlak@umk.pl*

The allometric growth rate of the alimentary tract segments with respect to the body mass was calculated in suckling, weaning and postweaning hamsters.

The study revealed that the body size of hamsters increased from birth to adulthood with the growth rate gradually decreasing with age.

The segments of the alimentary tract of hamsters grew simultaneously with the increase in the body size, although the rate of growth varied in time and space. The positively allometric increase in length of small and large intestine has been observed both during suckling and weaning period. The digestive-absorptive surface of segments of the alimentary tract, except the oesophagus, increased disproportionately quickly compared with the body mass from birth until complete weaning. After the weaning period, the growth rate of the internal surface of all segments was significantly slower and isometrically related to the growth of the body mass. This is because the surface area of mucosa in the stomach and small and large intestine of newborn hamster is disproportionately small compared with its size in adult specimens, and its essential growth occurred in the early postnatal period. These results suggest that obtain the adult proportion of the alimentary tract segments of hamster is an crucial condition for the proper growth an organism after the weaning.

Development of the ophthalmic artery in human embryos

Witold Woźniak, Małgorzata Bruska, Sławomira Fenger-Woźnicka, Mateusz Krajecki, Jarosław Sobański

Department of Anatomy, University of Medical Sciences, Święcickiego 6, 60-781 Poznań, Poland, e-mail: matejkrajecki@o2.pl

The literature concerning arterial supply to the region of the developing eye shows considerable diversity in both the nomenclature and identification of the vessels in this territory. The vascular system of the developing eye is established between weeks 4 and 8.

The aim of the study is description of the formation of definitive ophthalmic artery and its branches in staged human embryos between 5 and 8 weeks.

Investigations were performed in staged human embryos of developmental stages 13 to 23 (postovulantory age 32 to 56 days). Embryos were from collection of the Department of Anatomy in Poznań. Age of embryos was established according to international staging criteria. Serial sections made in sagittal, horizonatal, and frontal planes were stained with routine histological methods and impregnated with silver salts.

The first blood vessels appear at the time of invagination of the optic vesicle and formation of the optic cup. It was shown that in earliest investigated embryos aged 32 days (stage 13) the internal carotid artery and internal jugular vein are formed.

The internal carotid artery gives off the primitive dorsal ophthalmic artery, which extends to the optic cup. During stage 14 (embryos aged 33 days) develops the primitive ventral ophthalmic artery which is connected with the hyaloid artery arising from the internal carotid artery. Both ophthalmic arteries terminate in the plexus around the optic cup.

In embryos aged 39 and 41 days (developmental stages 16 and 17) the both primitive ophthalmic vessels elongate and the hyaloid branch to the lens originating from the dorsal ophthalmic artery develops. During the stage 19 (46 days) the permanent ophthalmic artery is established and the supraorbital branch arising from the stapedial artery develops. The ophthalmic artery gives off the ocular branches and the orbital branches of the supraorbital artery join the permanent ophthalmic artery.

At the end of embryonic period in embryos aged 53 to 56 days (stages 22 and 23) the ophthalmic artery acquires its adult form and its ocular and orbital branches are recognizable.

The development of the width and height of the orbital entrance

Witold Woźniak, Sławomira Fenger-Woźnicka, Małgorzata Grzymisławska, Magdalena Rojewska, Jarosław Sobański, Joanna Łupicka

Department of Anatomy, University of Medical Sciences, Święcickiego 6 Street, 60-781 Poznań, Poland, e-mail: rojewskamagdalena4@gmail.com, rojewskamagdalena4@gmail.com

Changes of measurements of the orbit during development depend on the growth of the eye and the skeleton of the face and the neurocranium between which the orbit is placed. The orbit lies laterally to the nasal cavity and development of the paranasal sinuses influences its growth.

The aim of the study was evaluation of the width and height of the orbital entrance in human fetuses. Investigations were performed in 50 human fetuses aged between 9 and 40 weeks. Fetuses fixed in 10% solution of formalin were from the collection of the Department of Anatomy University of Medical Sciences in Poznań. Measurements of the orbital entrance were made with vernier calipers. The width of the orbit was measured between frontmaxillary and frontozygomatic sutures. The height of the orbital entrance was the distance from the supraorbital notch to the lower margin of the orbit.

In early fetal period the bones forming the orbit are fibrous membranes with the ossification centers. It was shown that through the investigated period the width of the orbit exceeds its height. Both the width and the height measurements showed a linear development. The width ranged from 4.2 mm at 9 weeks to 25 mm at 40 weeks. The height ranged from 2.6 mm at 9 weeks to 23 mm at 40 weeks.

The height of the orbit increases due to development of the viscerocranium, particularly the development of the lateral wall of the nasal cavity which depends on the growth of the maxillary and ethmoidal sinuses. There were no differences in the width and height of the orbital entrance between the right and the left orbit.

Mandibulate moths (Micropterygidae) one of the most primitive family of Lepidoptera – ovary structure and oogenesis in comparison to other Lepidoptera and Trichoptera

Karol Żłobiński, Marta Mazurkiewicz-Kania

Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw, Sienkiewicza 21, 53-335 Wroclaw, Poland, e-mail: marta.kania@uni.wroc.pl

Micropterygidae are considered as one of the most primitive, archaic family of Lepidoptera. Like in other lepidopterans paired ovaries of *Micropterix cathella* (Micropterygidae) are of meroistic – polytrophic type. Each ovary is composed of five parallel ovarian tubes (ovarioles). In contrast to higher Lepidoptera, the ovarian tubes of *M. cathella* are relatively short and formed by a dozen of egg chambers. Each egg chamber consists of a cluster of eight sibling germ cells (seven nurse cells plus one oocyte) surrounded by a single layer of the follicular cells (FCs). In the course of oogenesis FCs becomes diversified into three subpopulations: 1) main body FCs (covering the lateral and basal sides of the oocyte), 2) FCs covering the nurse cells surface and 3) FCs encased at the anterior pole of the oocyte. The basis pattern of follicular cells diversifications seems to be similar in all examined Lepidoptera and Trichoptera. The significant difference in this process concerns the interfollicular stalk formation. For example the mechanisms of interfollicular stalk genesis are different in Sphingidae and Pieridae, whereas the presence of interfollicular stalks has been never found in the ovarioles of Micropterygidae.

In Micropterygidae the germ cells within egg chambers form branched clusters. The oocyte is directly connected by three intercellular bridges to three of its siblings (nurse cells). The nurse cells that directly adjoin the oocyte are significantly larger than the remaining ones. Our studies reveal that in all nurse cell's nuclei the extrachromosomal amplification of rDNA, manifested by presence of characteristic compact DNA – positive body or numerous nucleoli, takes place. Although the nurse cells support the oocyte growth, the germinal vesicle is not transcriptionally silent. The same situation was observed in trichopterans.

Micropterygidae and Trichoptera are characterized by the present of symbiontic microorganism in the cytoplasm of FCs and the oocyte. These bacterial endosymbionts are rod-shaped and limited by a double membrane. It is generally accepted that symbionts play an important physiological role providing the amino acids or vitamins to the host insects.

Taste organs during development of *Mantidactylus betsileanus* (Anura, Mantellidae)

Krystyna Żuwała[1], Karolina A. Budzik[1], Maciej Pabijan[1], Miguel Vences[2]

[1]*Department of Comparative Anatomy, Jagiellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: krystyna.zuwala@uj.edu.pl, karolina.kawa@uj.edu.pl*
[2]*Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany*

In previously investigated Anura and Caudata species with indirect development (i.e. with a larval stage) there are two generations of taste organs (e.g. Żuwała, 2005). According to a recent study (Budzik and Żuwała *in preraration*) the type of development (direct or indirect) has an impact on the number of generations of taste organs in amphibian ontogeny. Confirmation of this hypothesis requires further research of taste organ development, particularly in direct-developing amphibians, but also in other indirect-developing species. The latter include *Mantidactylus betsileanus* which is endemic to Madagascar. For observations of oral epithelium of *M. betsileanus* tadpoles (31–46 stage according to Gosner, 1960) scanning electron microscopy was used.

At stage 31 in the front part of the oral floor the tongue fold begins to develop, while the lining of the palate and oral floor forms numerous finger like papillae (FLP) with visible sensory zones of taste buds (TBs) on top. Numerous TB sensory zones are also visible on small mucosa elevations in the back part of the oral floor and in the central part of the palate. The tongue fold grows and elongates anteriorly and faucially. On its dorsal surface a second generation of taste organs – taste discs (TDs) – begins to form. At stage 45 TD sensory zones (surrounded by ciliated epithelial cells) are very well developed in the oral epithelium of the tongue and the palate. FLP and TBs, including those lying on the small elevations, are resorbed in the period between stages 42 and 45. At the end of metamorphosis (stage 45), the area of TD sensory zones of the tongue is 240 μm^2 on average and its diameter ranges from 16 to 22 μm. At stages 45 and 46 the areas of TD sensory zones in both the palate and the tongue varied.

Conclusion. The results of the present study of taste organ morphology of *M. betsileanus* confirms that there are two generations of taste organs in indirect-developing species.

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