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COMMEMORATIVE LECTURE

Mammalian interspecific chimaeras – In the memory of Professor Andrzej K. Tarkowski (1933–2016)

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In the last fifty years the interspecific adult chimaeras have been generated by different methods in several closely related mammalian species. However, mouse<->rat chimaeras able to develop to term have been especially difficult to obtain. In order to examine interactions between cells originating from different species during embryonic development we constructed mouse<->rat chimaeras by aggregation of 8-cell embryos which formed blastocyst in vitro, at which stage they were transferred into uteri of pseudopregant mice. We observed that shortly before implantation all primary cell lines of blastocysts became chimaeric. Majority of chimaeric mouse<->rat blastocysts implanted in mouse uterus, and out of those 46% developed into fetuses and pups, half of which were chimaeric. Although chimaeric animals were able to reach adulthood, high contribution of rat cells tended to diminish their viability (Bożyk et al., 2017).

Subsequently we tested the possibility of the development of pure (non chimaeric) mouse and rat fetuses in uteri of females of opposite species. To this goal we created chimaeric mouse<->rat blastocysts by injection of mouse embryonic stem cells (ESCs) into 8-cell rat embryos and rat ESCs into 8-cell mouse embryos to obtain chimaeric blastocysts in which only epiblast (embryonic cell line) was built of xenogenic ESCs. Chimaeras were transferred into foster mothers of the opposite species. We found that except one live fetus derived solely from the mouse ESCs, which was isolated at E13.5 from rat uterus, all other fetuses and newborns were chimaeric or were built only from the cell of recipient embryo. We conclude that in rat<->mouse model even when extraembryonic tissues of chimaeric embryo are composed solely of the cells of same species as the recipient female, the full-term development of the pure xenogenic fetus is very unlikely (Szpila et al., 2020).

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REFERENCES

Bożyk K, Gilecka K, Humięcka M, Szpila M, Suwińska A, and Tarkowski AK. 2017. Mouse<->rat aggregation chimaeras can develop to adulthood. *Development Biology* 427: 106–120.

Szpila M, Humięcka M, Bożyk K, Paterczyk B, Suwińska A, Maleszewski M, and Tarkowski AK. 2020. Attempts to obtain fully xenogenic fetuses in rat<->mouse model. *Biology of Reproduction* 102: 499–510.

PLENARY LECTURES

Embryonic cell cycle as viewed by biologist and mathematician

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The cell cycle of *Xenopus laevis* embryos is simplified and consists of S and M phases. Its biochemistry can be studied in cell-free extracts (Kubiak, 2016). We focus on the role of the CDC6 (Cell Divison Cycle 6) protein in regulating the first embryonic mitosis. We have shown that CDC6 acts as an inhibitor of the main enzyme initiating mitosis, namely CDK1 (Cyclin-Dependent Kinase 1) (El Dika et al., 2014). CDC6 determines not only the dynamics of CDK1 activation, but also the characteristic inflection of the activation curve during early stages of mitosis. This shape of the CDK1 activation curve resembles the growth curve of microorganisms under changing culture conditions called diauxic growth. CDC6 appears to be the only factor responsible for the diauxic increase in CDK1 kinase activity during mitosis (Borsuk et al., 2017). The mathematical description we propose concerns the relationship between proteins regulating the CDK1 activation system (Debowski et al., 2019). These are: CDK1 itself, the CDK1 regulatory subunit – cyclin B, CDC25 phosphatase and CDC6. Our model allows us to formulate a new hypothesis on the types of interactions between these proteins with CDK1 determining the correct, diauxic dynamics of CDK1 activation during mitosis. We also constructed a mathematical model explaining interactions between CDK1 and PP2A phosphatase during the same mitosis (Debowski et al., 2016). These two models will be presented as computer simulations visualizing the complexity of molecular interactions regulating the timing and progression of the first embryonic mitosis in *Xenopus laevis* embryo.

REFERENCES

Borsuk E, Jachowicz J, Kloc M, Tassan JP, and Kubiak JZ. 2017. Role of Cdc6 during oogenesis and early development in mouse and Xenopus. *Results and problems in cell differentiation* 59: 201–211.

Debowski M, El Dika M, Malejczyk J, Zdanowski R, Prigent C, Tassan JP, Kloc M, Lachowicz M, and Kubiak JZ. 2016. Flexibility vs. robustness in cell cycle control: mathematical model of interplay between CDK1, PP2A and CDC25 in regulation of timing of M-phase entry. *The International Journal of Developmental Biology* 60: 305–314.

Dębowski M, Szymańska Z, Kubiak JZ, and Lachowicz M. 2019. Mathematical model of the role of CDC6 in CDK1 activation upon M-phase entry. *Cells* 8(12).

El Dika M, Laskowska-Kaszub K, Koryto M, Dudka D, Prigent C, Tassan JP, Kloc M, Polanski Z, Borsuk E, and Kubiak JZ. 2014. CDC6 controls dynamics of the first embryonic M-phase entry and progression via CDK1 inhibition. *Developmental Biology* 396: 67–80.

Kubiak JZ. 2016. Cell-free extracts in Development and Cancer Research. *The International Journal of Developmental Biology* 60: 189–191.

Hybridogenesis through the eyes of a developmental and evolutionary biologist

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Hybridogenesis is one of specific modifications of reproduction of hybrids between closely related sympatric species. It has been described in one invertebrate (*Bacillus*) and seven vertebrate complexes (fish: *Poeciliopsis, Hexagrammos, Hypseleostris, Misgurnus, Squalius,* and amphibians: *Bufotes* and *Pelophylax*). Hybridogenetic hybrids overcome reproductive isolation mechanisms by a specific way of gametogenesis, in which chromosome set of one of the parental species is eliminated. This in turn enables to avoid incompatibilities in homologue pairing during meiosis, but at the expense of no genetic recombination and clonal gametes. In the eyes of evolutionary biologists, the absence of recombination is synonymous with asexual reproduction, which is difficult to accept by developmental biologists.

Hybridogenesis can be stated indirectly by genetic analyses of progeny obtained by backcrosses of hybrids and their parental species or directly by cytogenetic analysis of gametes. The former approach, with a focus on population genetics and speciation, is widely used by evolutionary biologists and results in the vast majority of publications. The latter one, which focuses on cytological mechanisms of genome elimination and gamete formation, is represented by developmental biologists and is extremely rare.

According to evolutionary biologists, there are two types of hybridogenesis: "mitotic" in diploid hybrids and "meiotic" in allotriploids. In diploid hybridogenesis one of the parental genomes is eliminated and the remaining one is copied by reduplication, whereas in triploids elimination concerns a single-copied genome. Because the remaining two chromosome sets usually represent different genetic lines, they recombine to some extent and the resulting gametes are not clonal. According to developmental biologists, each of these types of hybridogenesis is meiotic, because elimination (and endoreplication in diploids) is followed by regular meiosis.

Cytological mechanism of genome elimination has been described only in *Peciliopsis* and *Pelophylax*. However, the scenarios are different (one-pole mitosis in the former and formation of micronuclei in interphase in the latter). The main reason why there is so little research is lack of studies of very early development of gonads because elimination (and endoreplication) takes place only in gonocytes, not in differentiated germ cells. The best example is very rare male hybridogenesis in *Pelophylax*, in which gonocytes displays all states characteristic of hybridogenesis, while spermatogonial stem cells in adults have identical chromosome sets.

The parallel evolution of the maternal-fetal relationship in viviparous teleost fishes

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Viviparity, a highly successful mode of reproduction evolved more than 150 times among vertebrates (Blackburn, 2015). The first viviparous vertebrates were fishes: the chondrichthyans, the actinistians and the teleosts. Among teleost fishes, containing over 30 thousand species, the viviparity evolved independently 12 times. The nowadays living species exhibits wide scope of maternal-fetal interactions, involving the ovary for the embryonic maintenance, due to lack the Müllerian ducts (Wourms at al., 1988). Intraovarian gestation, which occurs either in the ovarian follicle and/or in ovarian lumen is preceded by the intrafollicular fertilization, which exclude ovulation before gamete fusion. The degree of teleost viviparity vary from strict lecithotrophy, with nutritional independency of the embryo relying on yolk reserves, to matrotrophy, in which nutrients for developing embryos are supplied by the mother (Meisner and Burns, 1997). The matrotrophy is unspecialized such as oophagy/adelphophagy or specialized, including extraordinary diversity of maternal (= ovarian) and embryonic tissues adaptations facilitating the nutrients transfer. The maternal tissues modifications involve epithelium lining ovarian lumen or ovarian follicle, forming such structures as vascularized secretory folds and embryonic tissues, such as: absorptive epithelium of gill, mouth or whole body surface, hypertrophied finfolds, pericardial sac, hindgut with a hypertrophied intestinal epithelium and/or its externalization forming trophothaeniae (Wourms et al., 1988). These various maternal and fetal tissues between which nutrient and gas exchange occurs, when lie in close apposition form different type physiological placentas called e.g."buccal" or "follicular placentas". The unique form in teleost viviparity is male incubation of developing embryos in pipefish and seahorses called male gestation or patrotrophic viviparity (Stölting and Wilson, 2007).

REFERENCES

Blackburn DG. 2015. Evolution of vertebrate viviparity and specializations for fetal Nutrition: A quantitative and qualitative analysis. *Journal of Morphology* 272: 961–990.

Meisner AD, and Burns JR. 1997. Viviparity in the halfbeak genera Dermogenys and Nomorhamphus (Teleostei: Hemiramphidae). *Journal of Morphology* 234: 295–317.

Stölting KN, and Wilson AB. 2007. Male pregnancy in seahorses and pipefish: Beyond the mammalian model. *BioEssays* 29: 884–96.

Wourms JP, Grove BD, and Lombardi J. 1988. The maternal-embryonic relationship in viviparous fishes. In: Hoar WS and Randall DJ [eds], 1–134. *Fish Physiology*, Vol. XI. *The Physiology of Developing Fish. B. Viviparity and Posthatching Juveniles*. San Diego, Academic Press, Inc.

Studying genome size, cell cycle and endoreduplication by flow cytometry

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Flow cytometry (FCM) is a method of measuring physical and/or chemical characteristics of single cells or their organelles, e.g. nuclei, mitochondria or chloroplasts, based on their optical properties. It was initially developed for analyzing bacteria and animal/human cells, particularly white blood cells (Baran, 2008). In the 1980s, after a simple and fast procedure to isolate nuclei from plant material has been reported, it started to be used commonly for estimations of nuclear DNA content in plants. The advantage of FCM is that is provides for easy and rapid sample preparations and precise measurements of DNA content in thousands of cells/nuclei in a short time. At present it is applied not only to plant research (biotechnology, taxonomy, cytogenetics, evolution, ecology) but also to plant breeding and seed production/technology. The most common application of FCM is estimation of ploidy and genome size, but it is also used for establishing cell cycle activity and endoreduplication intensity in different plant organs and tissues (Sliwinska, 2018). It can be used to analyze all kind of plant material (e.g. leaf, stem, seed, pollen, callus, embryos) if only it contains intact nuclei. It is applicable for analyzing plants grown in the field or greenhouse as well as in those cultured *in vitro*. Plant material grown in tissue culture is especially variable in its DNA content due to somaclonal variation; therefore, FCM analysis is strongly recommended to detect this during *in vitro* culturing. Estimation of nuclear DNA content is also necessary when polyploid plants are produced. FCM can be used to study mitotic cell cycle and endoreduplication since both are characterized by changes in nuclear DNA content depending on stage or intensity. Therefore, FCM analyses are performed to establish seed development, maturity, quality and advancement of germination, to follow embryo/seedling/plant development as well as to study cytotoxicity of some phytochemicals, e.g. those used in cancer therapy.

REFERENCES

Baran J. 2008. Nowa epoka cytometrii przepływowej – przewodnik po współczesnych cytometrach i ich zastosowanie. *Postępy Biologii Komórki* 35 Suppl. 24: 3–15.

Sliwinska E. 2018. Flow cytometry – a modern method for exploring genome size and nuclear DNA synthesis in horticultural and medicinal plant species. *Folia Horticulturae* 30: 103–128.

ORAL PRESENTATIONS

The process of spermatogenesis in the inseminating osteoglossiform *Pantodon buchholzi* (Pisces: Teleostei)

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Osteoglossomorpha is a basal Teleostei group with diverse biology of reproduction reflected by dominating external fertilization and occurrence of uniflagellate, biflagellate and aflagellate aquasperm versus internal fertilization present only in *Pantodon buchholzi*, being representative of the monotypic family Pantodontidae and producing the complex introsperm (Mattei, 1970; Deurs and Lastein, 1973; Dymek and Pecio, 2019; Mattei et al., 2019). These spermatozoa with conical nucleus and 9 helically arranged mitochondrial derivatives constituting ~55% of the total sperm length are condensed into sperm packets (Deurs and Lastein, 1973; Dymek and Pecio, 2019). The main aim of our study was to describe process of spermatogenesis using technics of electron microscopy (TEM, SEM). Primary spermatogonia have irregularly shaped nucleus located centrally, whereas late spermatogonia exhibit polarity in the arrangement of organelles (Golgi apparatuses, endoplasmic reticulum and mitochondria) and regularly shaped nucleus in the opposite poles. In the primary spermatocytes the prominent Golgi apparatuses start gradually degrading through compaction of the cisternae. They form electron dense round structures, which next transform into unique spindle-shaped bodies. Spermatid differentiation includes flagellum formation, chromatin condensation linked with nucleoplasm elimination and nuclear rotation, migration and fusion of mitochondria alongside the cytoplasmic canal. In elongated spermatid an excess of cytoplasm containing useless organelles and spindle-shaped bodies form residual bodies extruded into the cyst lumen. They are located peripherally within the cyst and phagocytised by Sertoli cells. In the final stage of spermiogenesis all extremely elongated spermatids are arranged parallel each other with heads on one pole and flagella on the other forming spermatozeugmata, which are later released into the lumen of the testicular tubules.

REFERENCES

Deurs B, and Lastein U. 1973. Ultrastructure of the spermatozoa of the teleost *Pantodon buchholzi* Peters, with particular reference to the midpiece. *Journal of Ultrastructure Research* 42: 517–533.

Dymek A, and Pecio A. 2019. Testis structure and sperm packet formation in the phylogenetically basal teleost *Pantodon buchholzi*. *Zoologischer Anzeiger* 279: 103–110.

Mattei X. 1970. Spermiogenèse comparée des poissons. In: Baccetti B [ed.], *Comparative spermatology*, 57–59. Academic Press, New York.

Mattei X, Marchand B, and Quilichini Y. 2019. A biflagellate spermatozoon in the African bonytongue *Heterotis niloticus* (Teleostei, Osteoglossidae). *Journal of Fish Biology* 94: 335–338.

Comparative studies of peripheral olfactory organ in basal Teleostei group, Osteoglossiformes (Pisces: Teleostei: Osteoglossomorpha)

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Osteoglossiformes is a very diverse group exhibiting numerous primordial features. Representatives of 5 families show variation e.g. in morphology and reproductive biology. Some of them are air-breathing fish what may suggest presence of adaptations to short ventures out of water (Graham, 1997). The main aim of the study was to describe structure and ultrastructure of olfactory organs of air-breathing *Pantodon buchholzi* (Pantodontidae), *Arapaima gigas* (Osteoglossidae) and *Gymnarchus niloticus* (Gymnarchidae) and water-breathing *Osteoglossum bicirrhosum* (Osteoglossidae) using light and electron (TEM, SEM) microscopy. Our studies indicate presence of olfactory rosette on the bottom of olfactory chamber of each studied species, however there are great differences in its composition among them. *P. buchholzi* possesses typical olfactory rosette composed of two rows of olfactory lamellae and centrally located elongated median raphe. In olfactory chamber there is also peculiar cudgel-shaped structure. In *A. gigas* olfactory lamellae are arranged semicircular and possess processes. They are joined to the short median raphe placed on the proximal wall of the olfactory chamber. *G. niloticus* has the olfactory rosette consisted of only short olfactory lamellae arranged in circle. In *O. bicirrhosum* there is also lack of median raphe, but the lamellae are arranged nearly parallel. The olfactory lamellae of each species are lined with olfactory epithelium divided into sensory and nonsensory compartments, however they are arranged in different fashion. The differences between species may reflect long lasting evolution of the families belonging to Osteoglossiformes and may suggest evolutionary trend in fish to simplification of the structure of the olfactory rosette, which could have appeared multiple times among different fish lineages.

REFERENCES

Graham J. 1997. Diversity and Natural History. In: Graham J [ed.], *Air-Breathing Fishes*, 13–63. Academic Press, California.

Caenorhabditis elegans vulva development as a screening platform for RTK-MAPK/ERK signaling inhibitors

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The RTK-MAPK/ERK pathway is an evolutionary conserved signalling pathway involved in cell proliferation, growth and differentiation (Zhang and Liu, 2002). It is estimated that 30% of human cancer patients present with activating mutation in the pathway. In melanoma, BRAF mutations are reported in up to 70% of the cases, with the *BRAF*^{V600E} mutation being the most common (Cantwell-Dorris et al., 2011). Treatment with BRAF inhibitors and combinations of BRAF and MEK inhibitors have revolutionized the clinical management of metastatic melanoma, but resistance development remains an obstacle for long term survival. Therefore, finding new inhibitors for RTK-MAPK/ERK-driven cancers is of great importance to the clinical management of melanoma in particular and to many other human cancers in general. Here, we aim to generate a *Caenorhabditis elegans*-based, whole-organism phenotypic screening platform and screen for novel inhibitors of the RTK-MAPK/ERK pathway. In *C. elegans*, aberrations in the pathway result in readily scored vulval phenotypes. When the pathway is over-activated, worms develop excess vulva tissue and display the so-called multivulva phenotype. On the other hand, inhibition of the pathway results in loss of vulva tissue and a vulvaless phenotype. In our lab we have generated *C. elegans* strains with activating RTK-MAPK/ERK pathway mutations, mimicking common mutations found in human cancers. Our work shows promising results for many clinically used RTK-MAPK/ERK signalling inhibitors and demonstrate the feasibility of the vulva development platform for large-scale inhibitor screens.

REFERENCES

Zhang W, and Liu HT. 2002. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. *Cell Research* 12: 9–18.

Cantwell-Dorris ER, O'Leary JJ, and Sheils OM. 2011. BRAF^{V600E}: Implications for Carcinogenesis and Molecular Therapy. *Molecular Cancer Therapeutics* 10: 385–394.

Biphasic respiration supports offspring development in viviparous dermapteran, *Arixenia esau* (Hexapoda, Dermaptera)

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Development inside a mother's body poses many physiological challenges for both the mother and her offspring (Ostrovsky et al., 2016). A recent study has indicated that, in a viviparous dermaptran *Arixenia esau*, embryonic development is separated into two phases: intraovarian and intrauterine (Tworzydlo et al., 2013). In an effort to comprehensively characterize viviparity in *Arixenia*, we aimed at elucidating the mechanisms of embryonic and larval respiration and gas exchange during the intrauterine phase of development. Our advanced microscopic analyses of maternal and embryonic/larval tissues as well as immunological approach, revealed that *Arixenia* evolved a unique biphasic system supporting offspring respiration while inside the mother's reproductive tract. This system relies on: 1/ an extensive network of trachea surrounding and penetrating the outer wall of the mother's uterus, 2/ distinct outgrowths located on the abdominal segments of advanced embryos and larvae tightly adjacent to the internal uterine wall (Bilinski and Tworzydlo, 2019), likely to participate in gas transferring, and 3/ respiratory pigment, hemocyanin, present within the body cavity of the larvae. Based on the obtained results, we propose that in the first phase of respiration, air/oxygen is supplied to the region of the mother/larva interface by the extensive maternal tracheal system associated with the uterus wall. Next, oxygen diffuses through the thin tracheole walls, passes the relatively thin tissue of the abdominal outgrowths and diffuses into the hemocyanin-enriched hemolymph of the larvae. In the second phase, the hemocyanin-bound oxygen is distributed throughout the larval body with circulating hemolymph.

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REFERENCES

Bilinski SM, and Tworzydlo W. 2019. Morphogenesis of serial abdominal outgrowths during development of viviparous dermapteran, *Arixenia esau* (Insecta, Dermaptera). *Arthropod Structure and Development* 49: 62–69.

Ostrovsky AN, Lidgard S, Gordon DP, Schwaha T, Genikhovich G, and Ereskovsky AV.2016. Matrotrophy and placentation in invertebrates: a new paradigm. *Biological Reviews* 91: 673–711.

Tworzydlo W, Kisiel E, and Bilinski SM. 2013. Embryos of the viviparous dermapteran, *Arixenia esau* develop sequentially in two compartments: terminal ovarian follicles and the uterus. *PLoS One* 8: e64087.

Male germ-line cysts in medicinal leeches from genus *Hirudo*

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Our knowledge about spermatogenesis of leeches from the genus *Hirudo* (six species: *H. medicinalis*, *H. verbana, H. orientalis, H. nipponia*, *H. troctina* and *H. sulukki*) is poor and there is no comparative analysis. Although, spermatogenesis has been already examined in *H. medicinalis* at the ultrastructural level more than 40 years ago, but in that time all European medicinal leeches (*H. medicinalis*, *H. verbana* and *H. orientalis*) were classified as *H. medicinalis*.

Specimens of *H. medicinalis*, *H. verbana, H. orientalis,* and *H. nipponia* were fixed and processed in accordance with the appropriate protocols. Light and transmission electron microscopy were used to analyze the general morphology of male reproductive systems and to analyze the structure and ultrastructure of germ cells. Fluorescence microscopy was used to analyze the distribution of the mitochondria and their activity and to visualize microtubular and F-actin cytoskeleton. Additionally, Serial Block Face Scanning Microscopy (SBEM) was applied to analyze the spatial (3D) conformation of mitochondria during consecutive stages of spermatogenesis.

The male reproductive system of medicinal leeches is composed of nine pairs of testes that are located intersegmentally on both sides of the body. Male germ cells inside the testis are united into syncytial groups of cells that are known as cysts, clones or clusters. Although the cysts are in different stages of development (thus, there is no synchrony between the cysts), all of the germ cells in a given cyst are at the same developmental stage. As in other clitellate annelids, the male germ-line cysts are equipped with a central mass of cytoplasm (cytophore) that occupies the center of each cyst, whereas the cells are arranged peripherally. The cells are interconnected to cytophore by specific cellular junctions that are called intercellular bridges (IBs). The IBs are elongated and had a cylinder-like shape and are rich in F-actin. Our analysis revealed the detailed spatial organization of microtubules, F-actin and mitochondria during consecutive spermatogenesis stages.

Adaptation for embryo nutrition in the pseudoscorpion *Chelifer cancroides*

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In animals, two modes of embryo nutrition have been evolved: lecithotrophy and matrotrophy. In the lecithotrophic type, the embryos are nourished with reserve materials deposited in the cytoplasm of the oocyte, while in the matrotrophic type the embryos are fed on nutrients produced by maternal tissues. In invertebrates matrotrophy has been described in all phyla. The prevailing majority of chelicerates is lecithotrophic, while matrotrophy is characteristic of scorpions and pseudoscorpions. In both matrotrophic chelicerate taxa the female gonads are involved in providing the nutrients for developing embryos. In scorpions, the female gonad termed ovariuterus fulfils the function of the uteri. The embryos develop either in the lumen of the ovariuterine tubules (nutrients for developing embryos pass from hemocoel through the ovariuterine wall) or in the ovariuterine diverticula (the embryos are nourished with the material produced in the hepatopancreas and provided to the embryo via a feeding apparatus). In pseudoscorpions, the embryos develop in the brood sac carried by the female on the abdominal site of the opisthosoma. It has been widely accepted that the nutritive fluid for developing embryos is produced by the ovaries and absorbed by the embryos via a pumping organ. Our studies aimed to show the structure of the female reproductive system during secretory phase of the ovarian cycle in the pseudoscorpion *Chelifer cancroides*. According to Weygoldt (1969) *Chelifer* is specialized for the extraembryonic nutrition due to synchronization in synthesis of the nutritive fluid and development of the pumping organ in the embryo body. We showed that in *Chelifer* the nutritive fluid for developing embryos is produced coordinately in the ovaries and the oviducts by the polyploid and hypertrophic epithelial cells. The results of our study clearly indicate that in *Chelifer* the efficiency of the nutritive fluid production is very high and so *Chelifer* is more specialized for the extraembryonic nutrition that it was previously believed. We also showed that the secretion of the nutritive fluid starts in early stages of embryogenesis. Therefore, we hypothesize that *Chelifer* embryos are able to absorb the nutritive fluid before formation of the pumping organ.

REFERENCES

Weygoldt P. 1969. *The Biology of Pseudoscorpions*. Harvard books in biology, no. 6. Harvard University Press.

Elimination and endoreplication of chromosomes during spermatogenesis in diploid and triploid hybridogenetic water frogs *Pelophylax esculentus*

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Among all hybrid animals males are, as a rule, absent or infertile. The exception is the Palearctic water frog complex composed of two species: *Pelophylax lessonae* and *P. ridibundus* and their interspecific hybrids *P. esculentus* represented by diploid (RL) and triploid (RRL, RLL) individuals of both sexes. Hybrids perpetuate themselves by hybridogenesis. Modified gametogenesis in diploids involves elimination of chromosomes of one of the parental species and endoreduplication of the remaining genome, which enables meiosis entry. In triploid hybrids, the same status is achieved after single-copied genome elimination. We assumed that elimination of chromosomes takes place only once in a lifetime in gonocytes (G) of developing testes. Subsequently in subadults gonocytes transform into spermatogonial stem cells (SSCs) in which the genome is already eliminated. To confirm our hypothesis, we analyzed chromosomal compositions in germ line cells from undifferentiated gonads (G) in tadpoles until sexually mature testes (SSCs). Species-specific *P. ridibundus* probes were used for genome recognition in mitotic and interphase cells from squashes of gonads.

Our results show that before sexual differentiation the prevailing portion of gonocytes did not exhibit genome elimination and contained both parental genomes. After differentiation the number of cells showing genome elimination, endoreplication and aneuploidy increased. In adults, SSCs showed mainly genomic compositions following the hybridogenesis model: diploid RR genome after removal of L genome in diploid RL and triploid RRL males, either diploid LL genome in triploid RLL males. Surprisingly, numerous cells possessed chromosomal sets inconsistent with the rules, showing no elimination or duplication of initial genome, while others were haploid, polyploid and aneuploid. Spermatozoa represented the predominant fraction of haploid R (in RL and RRL), or L genome (in RLL). In addition, we found spermatozoa inconsistent with hybridogenetic rules and aneuploid, being probably the meiotic products of nontypical SSC's. Experimental crossings evidenced that some diploid and all triploid males transmitted haploid R or L genome and produced viable progeny; aneuploid spermatozoa were evidently not functional.

Diversity of symbiotic systems in issid planthoppers (Hemiptera, Fulgoromorpha)

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Since the diet of planthoppers does not contain essential amino acids, these insects harbor obligate symbionts (bacteria, yeast-like symbionts) which are responsible for synthesis of missing nutrients. These intracellular symbiotic microorganisms are localized in the cytoplasm of large, polyploid cells called bacteriocytes which aggregate forming large, elongated bacteriomes.

Most planthoppers examined so far are host to two symbiotic microorganisms: bacteria *Vidania* (Proteobacteria: Betaproteobacteria) and *Sulcia* (Bacteroidetes). It is hypothesized, that these bacteria represent ancestral symbionts that infected a common ancestor of planthoppers and codiversified with most lineages of Fulgoromorpha (Urban and Cryan, 2012).

Histological, ultrastructural and molecular studies were conducted on four planthopper species: *Issus coleoptratus, Scorlupella discolor, Tshurtshurnella decempunctata* and *Zopherisca tendinosa*. Microscopic observations revealed that in the body of all examined insects *Vidania* bacteria occur. *Vidania* bacteria are localized both in the cytoplasm of bacteriocytes within the bacteriomes and in bacteriocytes within the rectal organ which is located in the invagination of the hindgut epithelium. In *S. discolor, T. decempunctata* and *Z. tendinosa* apart from *Vidania*, the *Sulcia* bacteria and *Sodalis* bacteria (Proteobacteria: Gammaproteobacteria) were observed. In *T. decempunctata* and *Z. tendinosa* both *Sulcia* and *Sodalis* inhabit elongated bacteriomes. In individuals of *S. discolor Sulcia* bacteria occupy the bacteriomes, whereas *Sodalis* bacteria are localized in fat body cells and in the cytoplasm of bacteriocytes with *Vidania*. In *I. coleoptratus*, *Vidania* bacteria are associated with yeast-like symbionts which are distributed in fat body cells.

The obtained results indicated that all types of symbionts are transovarially transmitted to the next generation. In mature females, bacteria/yeast-like symbionts leave the bacteriocytes/fat body cells and accumulate around the follicular epithelium near the posterior pole of terminal oocytes. The symbionts migrate across follicular epithelium, then they accumulate in the perivitelline space and form “a symbiont ball” in the deep depression of the oolemma.

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REFERENCES

Urban J, and Cryan J. 2012. Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea). *BMC Evolutionary Biology* 12: 87.

Pancreas differentiation in grass snake *Natrix natrix* and sand lizard *Lacerta agilis*

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Pancreas is an organ which differentiates at similar mode in all vertebrate species. However, some specific differences in pancreatic development can be found not only among different groups of vertebrates but also within these groups. Differentiation of pancreas in reptiles, especially squamates, is poorly known. The aim of this study was to compare differentiation of pancreas in two representants of squamates – *Natrix natrix* and *Lacerta agilis*. Age of embryos, isolated at regular intervals, was calculated using developmental table (Peter, 1904; Rupik, 2002). Pancreatic tissue were prepared for light microscopy according to standard histological procedures. Serial histological sections were used for obtained three-dimensional reconstructions of pancreas and surrounding organs. Results of this study revealed that in both studied species pancreas was found for the first time just after egg-laying in small distance to liver primordium. During embryonic development shape of the gland changed. At time of hatching pancreas of grass snake was compact and elongated. In contrast, mature pancreas of sand lizard was triangular in shape and contained three processes. Pancreas in *Natrix natrix* was found in close proximity to gall bladder whereas location of gall bladder in sand lizard was in far distance from pancreas. In grass snake pancreatic islets were found only in the dorsal part of gland in close proximity to spleen, on the other hand, pancreatic islets in *Lacerta agilis* were observed in entire pancreatic gland. Moreover, pancreatic islets in *Lacerta* were smaller and more irregular in shape than islets in *Natrix* pancreas. In addition, some similarities could be observed between embryonic pancreas of studied representants of lizard and snake. In both grass snake and sand lizard the largest agglomerations of pancreatic islets were located in close proximity to the spleen anlage.

REFERENCES

Peter K. 1904. Normentafel zur Entwicklungsgeschichte der Zauneidechse (*Lacerta agilis*). In: Keibel F. [ed.], *Normentafeln zur Entwicklungsgeschichte der Wirbeltiere*. vol. 4. Gustav Fischer, Jena.

Rupik W. 2002. Early development of the adrenal glands in the grass snake *Natrix natrix* L. (Lepidosauria, Serpentes). *Advances in Anatomy, Embryology and Cell Biology* 164: 1–102.

Morphology and histology of embryonic retina in the brown anole, *Anolis sagrei* (Squamata: Iguania)

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The brown anole (*Anolis sagrei*) is diurnally active, visually-oriented lizard possessing an excellent, high-acuity visual system. Retina of this species is similar to described in the other diurnal non-ophidian squamates (Ali, 1960). During differentiation of the brown anole retina simple pseudo-stratified neuroepithelium was divided into different cell types forming seven layers. Retinal morphogenesis in the brown anole embryos was analyzed with the use of histological and scanning electron microscopic methods. The anole embryos were isolated at regular intervals, starting at eggs lying and finishing at hatching of the first individuals. The age of embryos was calculated using the table for the lizard genus *Anolis* (Sanger et al., 2008). Results of this study indicated, that at the beginning of embryonic development the largest part of differentiating retina was composed of a pseudo-stratified columnar epithelium with no apparent morphological signs of differentiated neurons. After half of embryonic development photoreceptor cells start to differentiate. Moreover, other retinal cells differentiate just before hatching.

REFERENCES

Ali MA. 1960. Observations on the retina of Florida chameleon (*Anolis carolinensis*). *Canadian Journal of Zoology* 38(5): 965–971.

Sanger TJ, Losos JB, and Gibson-Brown JJ. 2008. A Developmental Staging Series for the Lizard Genus *Anolis*: A New System for the Integration of Evolution, Development, and Ecology. *Journal of Morphology* 269: 129–137.

Patterns of growth, brooding and offspring size in the invasive mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) from an anthropogenic heat island

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In freshwater temperate environments, waterbodies warmed by power plants can serve as a stepping stone in the spread of tropical and subtropical species. This offers a unique opportunity to acquire an insight into the life history of organisms undergoing range expansions beyond their native habitat. Here, we studied the patterns of offspring production and growth in the Asian mussel *Sinanodonta woodiana* inhabiting the thermal plume of the Odra River in Central Europe. Compared to the *S. woodiana* males, females had more convex shells and had a greater tendency to continue post-maturation growth and thus follow an indeterminate growth pattern. Gravid females were observed all-year round, and individuals with either large or more convex shells brooded more offspring. The proportion of incubating females, brood size and glochidia size were linked to gonadal activity and changed seasonally. Females with a higher amount of nutritive substances in the ovaries produced more and larger offspring. The smallest and the largest offspring occurred in the summer and in the winter, respectively. *Sinanodonta woodiana* incubated different offspring generations simultaneously, constantly releasing them into the environment. Using a life history evolution perspective, we discuss our results in view of allocation trade-offs between growth and offspring production.

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REFERENCES

Labecka, AM, and Czarnoleski, M. 2019. Patterns of growth, brooding and offspring size in the invasive mussel *Sinanodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) from an anthropogenic heat island. *Hydrobiologia*, https://doi.org/10.1007/s10750-019-04141-9.

Expression of calnexin in *Petunia* germinating pollen and growing pollen tubes

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The sperm cells of angiosperms are immobile, thus they are delivered to the ovule by growing pollen tube. Polar growth of the pollen tube is regulated by calcium ions (Ca^{2+}) which form a tip-focused gradient in the tube cytoplasm. We hypothesize that two Ca^{2+}-binding chaperones of the endoplasmic reticulum (ER), calreticulin (CRT) and calnexin (CNX), are involved in the molecular mechanism of forming and maintaining of this Ca^{2+} gradient. We showed that in germinating pollen and growing pollen tubes of *Petunia hybrida* (*Ph*), CRT is translated on the ER-bound ribosomes while a post-transcriptional *PhCRT* gene silencing disrupts pollen tube growth (Suwińska et al., 2015, 2017). It has been also suggested that CNX plays a role in *Arabidopsis* pollen development and the tube growth (Vu et al., 2017). To address this possibility, we cloned and characterized the full-length cDNA of a new *PhCNX* gene. The deduced amino-acid sequence of the *Ph*CNX shares homology with other known plant CNXs. Using fluorescent *in situ* hybridization (FISH) we revealed distribution of *PhCNX* mRNAs in *in vitro* germinating pollen and growing tubes. In addition, our immunocytochemical experiments show that *Ph*CNX is localized to the apertures of germinating pollen and to the subapical zones of elongating tubes. Thus, our results support the idea that CNX (probably together with CRT) may have a role in molecular chaperoning during pollen germination and pollen tube elongation.

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This work was supported by the Polish National Science Centre, grant MINIATURA 2017/01/X/NZ3/00255.

REFERENCES

Suwińska A, Wasąg P, Zakrzewski P, Lenartowska M, and Lenartowski R. 2017. Calreticulin is required for calcium homeostasis and proper pollen tube tip growth in *Petunia*. *Planta* 245: 909–926.

Suwińska A, Lenartowski R, Smoliński Dj, and Lenartowska M. 2015. Molecular evidence that rough endoplasmic reticulum is the site of calreticulin translation in *Petunia* pollen tubes growing *in vitro*. *Plant Cell Reports* 34: 1189–1199.

Vu Kv, Nguyen Nt, Jeong Cy, Lee Y-h, Lee H, and Hong S-w. 2017. Systematic deletion of the ER lectin chaperone genes reveals their roles in vegetative growth and male gametophyte development in *Arabidopsis*. *The Plant Journal* 89: 972–983.

Symbiosis in planthoppers from Dictyopharidae family (Hemiptera, Fulgoromorpha) – variations in types of symbionts and modes of their transovarial transmission

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Planthoppers are phloem-feeding insects that live in symbiotic associations with bacterial or/and fungal microorganisms which supply them with essential nutrients deficient in their diet. Previous data revealed that ancestral, heritable symbionts of Fulgoromorpha (planthoppers) are bacteria *Sulcia* and *Vidania* (Urban and Cryan, 2012). However, in some families both symbiont loss and replacement took place. We investigated the symbiotic systems of seven planthopper species belonging to Dictyopharidae family: *Dictyophara pannonica*, *Dictyophara multireticulata*, *Dictyophara europaea*, *Parorgerius platypus*, *Ranissus scytha*, *Ranissus edirneus* and *Callodictya kruperi*. Amplicon sequencing and molecular cloning of 16S rRNA genes of symbionts revealed that all species examined are host to bacteria *Sulcia*, *Vidania* and one additional bacterial symbiont. *D. pannonica*, *D. multireticulata*, *C. kruperi* and *R. edirneus* harbor bacteria *Sodalis*, whereas *D. europaea*, *P. platypus* and *R. scytha* – bacteria *Arsenophonus*. Our histological and ultrastructural analyses showed that all these symbionts are localized in the cytoplasm of bacteriocytes that form separate bacteriomes. Besides bacteriomes bacteria *Vidania* occupy also bacteriocytes in the rectal organ which is localized in the invagination of hindgut epithelium.

All types of symbionts are transovarially transmitted between generations. However, we observed important differences in the mode of transmission of bacteria *Sodalis* and *Arsenophonus*. In all species examined bacteria *Sulcia* and *Vidania* infect the posterior end of the ovariole. They migrate to the perivitelline space via follicular epithelium. Bacteria *Arsenophonus* are always transmitted together with ancestral symbionts, whereas bacteria *Sodalis* may be inherited in different ways: (1) they may invade the neck region of the ovariole or (2) they may infect the posterior end of the ovariole together with bacteria *Sulcia* and *Vidania*.

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REFERENCES

Urban J., and Cryan J. 2012. Two ancient bacterial endosymbionts have coevolved with the planthoppers (Insecta: Hemiptera: Fulgoroidea). *BMC Evolutionary Biology* 12: 87.

Transovarial transmission of symbionts in scale insects (Hemiptera, Coccomorpha)

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It is generally known that symbionts of scale insects: (1) belong to different taxa of bacteria (Bacteroidetes, Proteobacteria) or fungi, (2) are distributed in different organs (in bacteriomes, in fat body, in midgut epithelium), (3) are in different way transmitted between generations (Baumann, 2005; Szklarzewicz and Michalik, 2007). In *Orthezia urticae* (Ortheziidae), *Matsucococcus pini* (Matsucococcidae), *Steingelia gorodetskia* (Steingeliidae) as well as in *Acanthoccus aceris* and *Gossyparia spuria* (Eriococcidae), symbiotic bacteria are localized in fat body cells. Symbionts of *Greenisca brachypodii* (Eriococcidae), *Puto superbus* (Putoidae) and all so far examined members of Pseudococcidae and Diaspididae are harbored in specialized cells of host insect termed bacteriocytes. Symbionts of members of family Kermesidae and Coccidae are fungi related to entomopathogens *Cordyceps* or *Ophiocordyceps* which are harbored in fat body cells.

In scale insects, like in other hemipterans, the beginning of the symbiont transmission from mother to her offspring is corelated with the course of oogenesis. Symbiotic microorganisms become released from cells of the fat body or bacteriocytes and start to migrate towards ovaries. In Ortheziidae, Matsucoccidae and Steingeliidae symbionts infect undifferentiated germs cells (cystocytes) in larval ovaries. In the adult females these microorganims migrate via trophic core and nutritive cords from trophocytes into the developing oocyte. In Eriococcidae, Putoidae, Pseudococcidae, Coccidae and Diaspididae ovaries of adult females are invaded. Symbionts (or whole intact bacteriocytes in *P. superbus*) enter the cytoplasm of follicular cells surrounding the nutritive cord or journey between neinghboring follicular cells. After crossing the follicular epithelium symbionts accumulate in the deep invagination of oolemma at the anterior pole of the oocyte.

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This work was supported by the Iuventus Plus V grant IP2015050374 from the Ministry of Science and Higher Education.

REFERENCES

Baumann P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sup-sucking insects. *Annual Review of Microbiology* 59: 155–189.

Szklarzewicz T, and Michalik A. 2017. Transovarial transmission of symbionts in insects. In: Kloc M [ed.], *Results and Problems in Cell Differentiation,* 43–67. Vol. 63*, Oocytes. Maternal Information and Functions,* Springer, Cham.

The spatial and temporal distribution of poly(A) RNA in female and male gametes of *Arabidopsis thaliana* and *Hyacinthus orientalis* L.

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Unlike animals, studies focused on cellular and molecular mechanisms involved in the process of sexual the flowering plants reproduction are much less advances. It is caused, first of all, by technical difficulties. In most angiosperms, sperm cells are formed in the pollen tube growing within the pistil, whereas the egg cell and the central cell are closed in the embryo sac and surrounded with many layers of somatic cells of the ovule and the pistil. Such a location makes it difficult for cytological and molecular examinations of reproduction cells and the mechanisms which occur during the fertilization.

Our previous reports, including the chromatin organization and the transcriptional activity in *Hyacinthus orientalis* (monocots) and *Arabidopsis thaliana* (dicotyledons) embryos sac cells indicate that in the egg cells and the central cell the chromatin is largely dispersed but their nuclear metabolism are different. In *H. orientalis* the both gametes are silenced while in *Arabidopsis* the pattern of transcriptional activity was different and probably related with the maturation of cells. In turn, our studies of hyacinth male gametes have shown the metabolic silencing of the sperm cells in which strongly condensed chromatin was observed.

In the light of these data the aim of our study was to determine the distribution of poly(A) RNA in *H. orientalis* and *A. thaliana* female and male gametes The fluorescence *in situ* hybridization (FISH) technique has not only allowed us to show the differences in the pattern of the localization of poly(A) RNA in the gametes but also has revealed a physical relationship between sperm cells and the vegetative nucleus within male germ unit-MGU in the tricellular pollen grain of *Arabidopsis* and after the generative cell division in growing pollen tube in hyacinth. We also observed the unique localization of poly (A) RNA in the egg cell and the central cell within female germ unit-FGU in both species. We confirm that the level of poly(A) RNA was positively correlated with the transcriptional activity of female and male gametes and discuss with the different chromatin organizations and epigenetic mechanisms of their expression.

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The conjugates of nanodiamonds and *Neb*-colloostatin or its hemocytotoxic analogues impair an ovarian function in the *Tenebrio molitor* beetle

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Neb-colloostatin, the insect ovarian peptide with a pleiotropic activity in insects and its more potent analogues can limit the reproduction of the insects (Czarniewska et al., 2014; Czarniewska et al., 2012; Kuczer et al., 2013). Recently, we have shown that *Neb*-colloostatin in conjugate with nanodiamond can transmigrate across the cuticle to hemocoel of the insect and inhibit cellular and humoral immune response in larvae, pupae and adult of the *Tenebrio molitor* (Czarniewska et al., 2019).

In this study, we demonstrated that the most active analogues of *Neb*-colloostatin, when conjugated with nanodiamonds, passed through the cuticle of *T. molitor* and induced changes in reproduction of the insect. They potently inhibited an ovarian development, reduced the number of eggs laid and larval hatchability. The identification of the new action of *Neb*-colloostatin analogues suggests the possibility of using these pleiotropic synthetic peptides as non-toxic and specific insecticidal agents.

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REFERENCES

Czarniewska E, Rosiński G, Gabała E, and Kuczer M. 2014. The natural insect peptide *Neb*-colloostatin induces ovarian atresia and apoptosis in the mealworm *Tenebrio molitor*. *BMC Developmental Biology* 14: 1–10.

Czarniewska E, Mrówczyńska L, Kuczer M, and Rosiński G. 2012. The pro-apoptotic action of the peptide hormone *Neb*-colloostatin on insect haemocytes. *The Journal of Experimental Biology* 215: 4308–4313.

Czarniewska E, Nowicki P, Kuczer M, and Schroeder G. 2019. Impairment of the immune response after transcuticular introduction of the insect gonadoinhibitory and hemocytotoxic peptide *Neb*-colloostatin: A nanotech approach for pest control. *Scientific Reports* 9: 1–12.

Kuczer M, Czarniewska E, Rosiński G, and Lisowski M. 2013. The pro-apoptotic action of new analogs of the insect gonadoinhibiting peptide *Neb*-colloostatin: Synthesis and structure-activity studies. *Peptides* 44: 149–157.

The conjugates of nanodiamonds and *Neb*-colloostatin or its analogues impair the immunity of the *Tenebrio molitor* beetle during development

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Neb-colloostatin, the gonadoinhibitory and hemocytotoxic peptide and its more active hemocytotoxic analogues are considered as peptides that may be used as bioinsecticides (Czarniewska et al., 2012; 2014; Kuczer et al., 2013). We have shown that *Neb*-colloostatin coupled to nanodiamonds can migrate through cuticle and reduce the immune response of *T. molitor* (Czarniewska et al., 2019).

In this study, we showed that four the most hemocytotoxic *Neb*-colloostatin analogues conjugated with nanodiamonds passed through the insect cuticle maintaining biological activity. When introduced into hemolymph, they caused a reduction in the number of phagocytes, a decrease in nodule formation and phenoloxidase activity in the experimentally infected larvae, pupae and adults. A peptide-induced decrease in the insect's immunity during metamorphosis may have significant negative consequences for its development and viability. The result of the action of these synthetic analogues may be a reduction in the number of insects in the population. The identification of new synthetic peptides simultaneously disrupting the functioning of many important life functions of the insect is of great importance for the development of environmentally safe peptidomimetics that can be used in pest control.

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REFERENCES

Czarniewska E, Rosiński G, Gabała E, and Kuczer M. 2014. The natural insect peptide *Neb*-colloostatin induces ovarian atresia and apoptosis in the mealworm *Tenebrio molitor*. *BMC Developmental Biology* 14: 1–10.

Czarniewska E, Mrówczyńska L, Kuczer M, and Rosiński G. 2012. The pro-apoptotic action of the peptide hormone *Neb*-colloostatin on insect haemocytes. *The Journal of Experimental Biology* 215: 4308–4313.

Czarniewska E, Nowicki P, Kuczer M, and Schroeder G. 2019. Impairment of the immune response after transcuticular introduction of the insect gonadoinhibitory and hemocytotoxic peptide *Neb*-colloostatin: A nanotech approach for pest control. *Scientific Reports* 9: 1–12.

Kuczer M, Czarniewska E, Rosiński G, and Lisowski M. 2013. The pro-apoptotic action of new analogs of the insect gonadoinhibiting peptide *Neb*-colloostatin: Synthesis and structure-activity studies. *Peptides* 44: 149–157.

A role of E-cadherin (*Cdh1*) and N-cadherin (*Cdh2*) in the mouse gonad development

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The development of gonad is critical for the sexual development and reproduction of the individual. During development, the bipotential gonad, which is an unorganized aggregate of cells, differentiates into highly structured testis or ovary. Cell adhesion molecules (CAMs) are a group of proteins key for the segregation and gathering of different cell types to establish a structure of tissues and organs. E-cadherin (*Cdh1*) and N-cadherin (*Cdh2*) are CAMs highly expressed in the developing gonads. We used tissue-specific knockout of *Cdh1* and *Cdh2* genes in OCT4+ germ cells and, separately, in SF1+ somatic cells of mouse developing gonads. The knockout of E-cadherin in somatic cells caused decrease in the number of germ cells, while the knockout in the germ cells caused their almost complete loss. Thus, the expression of E-cadherin in both the germ and somatic cells in developing gonad is necessary for the survival of germ cells. The differentiation of testis cords and steroidogenic fetal Leydig cells was not disrupted when E-cadherin was deleted in somatic or germ cells. The knockout of N-cadherin in somatic and germ cells led to the decreased number of germ cells. However, the knockout of N-cadherin in somatic cells additionally resulted in disruption of testis cords and decreased number of steroidogenic fetal Leydig cells. Gene expression analysis showed that N-cadherin promotes cell survival via DCR2 receptor inhibiting pro-apoptotic DR5 receptor, however, E-cadherin promotes PI3K pathway upregulating anti-apoptotic AKT kinase. Altogether, these results show that E- and N-cadherin are crucial for survival of germ cells, and N-cadherin is also important for maintenance of gonadal structure and steroidogenesis. Moreover, this study reveals that cadherins are responsible not only for cell adhesion but also for regulation of cell survival in development due to apoptosis regulation.

The influence of hybridogenesis on germ cells morphology in the testes of juvenile and adult *Pelophylax esculentus* water frogs

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The formation of functional germ cells in the hybrid water frogs *Pelophylax esculentus* is possible due to the process of hybridogenesis. The process of genome elimination and subsequent replication is imprecise and leads to the formation of aneuploid and polyploid cells, which then degenerate. The high proportion of abnormal cells significantly reduce the male's ability to produce functional gametes and thus affect fertility. Our goal was to check at which moment of testicular development and how frequently defective gonocytes appear, and whether spermatogonial stem cells (SSC) with abnormal genomic composition can lead to sperm formation.

We focused on the observation of morphology and size measurement: 1/ gonocytes in juvenile individuals, from undifferentiated gonad to metamorphic climax; 2/ spermatogonial stem cells (SSCs), meiocytes and spermatozoa in sexually mature diploid and triploid males. We also studied spermatozoa produced by sexually mature males (functional that result in viable offspring and abnormal).

In male gonocytes just after sexual differentiation of gonads, elimination of one of the chromosome sets via micronuclei was observed, simultaneously with gonocytes abnormally formed nucleus, two nuclei or multinucleated. In testes during metamorphic climax, the frequency of degenerating and gonocytes increased.

In adult male gonads, micronuclei were no longer observed in SSCs, but we still found cell nuclei disorders. Abnormally large SSCs were observed in both diploid (21.01–69.31%) and triploid individuals (11.32–52.68%), concomitant with SSCs degeneration. Single large meiocytes or whole cysts of large meiocytes were present in seminiferous tubules, resulting in formation of large spermatozoa. Cytogenetic studies have shown that large SSCs and meiocytes had multiplied genome (4N, 6N and 8N) and often were aneuploid. Three males produced more than one type of gametes (haploid and diploid). Histological examination of whole testes revealed that individuals with a large proportion of large SSCs had fewer meiocyte cysts and fewer spermatozoa resulted in abnormal morphology of testes. Moreover, the analysis of the chromosome composition of the offspring showed that only haploid R or L and occasionally diploid RL gametes were functional and led to the formation of viable tadpoles.

Morphogenesis and functions of the Balbiani body in the oocytes of Hemimetabola

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Early oocytes of various animal species contain transient non-membrane-bound organelle complex termed the Balbiani body (Bb). The Bbs always comprise two essential elements: numerous mitochondria and irregular accumulations of granulo-fibrillar nuage material. One of the suggested functions of the Bb is a transfer of mitochondria to the germ plasm, and consequently their transmission to the next generation. In apterygotous insect, *Thermobia domestica* mitochondria of the Bb form an extensive network. The membrane potential of mitochondria within the network is higher than the membrane potential of individual ones in the ooplasm. Moreover, the mitochondrial network of the Bb is very often surrounded by small isolated mitochondria which apparently degenerate. In the light of these findings we suggest that, at least in some species, the Bb is implicated not only in the transmission of mitochondria, but also in selective elimination of defective mitochondrial units. To verify this hypothesis we performed the ultrastructural and molecular analyses of the Bb in the oocytes of bush cricket, *Metrioptera brachyptera* (Tettigoniidae).

The results show that in tettigoniids the Bbs arise in early previtellogenic oocytes due to gradual aggregation of mitochondria and nuage material. Fully developed cap-shaped Bb consists of two zones: perinuclear and cytoplasmic. In the perinuclear zone numerous polymorphic accumulations of nuage, small bean-shaped mitochondria, and elements of endoplasmic reticulum are present. In the cytoplasmic zone, mitochondria cluster around large nuage accumulation forming characteristic nuage/mitochondria complexes. The mitochondria remaining in a direct contact with nuage accumulations elongate, bifurcate and multiplicate,_forming eventually local micro-networks. In contrast, the mitochondria not directly associated with the nuage often show signs of degeneration. As oogenesis progresses the nuage/mitochondria complexes are partitioned into progressively smaller entities that move towards oocyte cortex and consequently populate the whole oocyte cytoplasm.

In the light of our results, we suggest that in tettigoniids, as in *Thermobia* the Bb is involved in multiplication and selective propagation of ,,healthy" mitochondria to the offspring. Moreover, we speculate that multiplication of mitochondria might be initiated by the nuage material.

Do both proteins of the CNX/CRT cycle are crucial for pollen tube growth?

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Pollen tube is the fastest growing plant cell that transports two sperm cells to the embryo sac for double fertilization. Proper elongation of the tube requires precise regulation of calcium ions (Ca^{2+}) level in its cytoplasm, which determines the interaction of basic cellular processes such as exo/endocytosis and the intracellular transport of organelles and vesicles carried out with the cytoskeleton. The molecular mechanism stabilizing a tip-focused Ca^{2+} gradient in growing pollen tube has not been clarified to this day. The results of our studies indicate that calreticulin (CRT), a prominent Ca^{2+}-binding/buffering endoplasmic reticulum (ER)-resident chaperone, is a key element of the molecular mechanism controlling Ca^{2+} homeostasis in the growing tube. We demonstrated that post-transcriptional gene silencing of *Petunia hybrida CRT1/2* (*PhCRT1/2*) expression by exogenous small interfering RNA, strongly impairs Ca^{2+} gradient in elongated tube, leading to inhibition of its growth (Suwińska et al., 2017). Since CRT interacts closely with the other chaperone – calnexin (CNX) – creating a quality-control system of polypeptides in the ER (so-called CNX/CRT cycle), the role of CNX in pollen germination and pollen tube elongation can be equally important. Our initial studies show that *CNX* gene is expressed in *Petunia* germinating pollen and growing tubes. Using fluorescent *in situ* hybridization (FISH) and immunofluorescence techniques we revealed distributions of *Ph*CNX mRNA and *Ph*CNX protein, respectively. In germinating pollen grains both *CNX* transcripts and the protein accumulate in the cytoplasm of the pollen apertures while in elongating tubes these transcripts and CNX were localized mostly in the subapical zones. Thus we argue that the transmembrane CNX together with its soluble paralogue CRT, may be crucial for proper pollen tube growth.

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REFERENCES

Suwińska A, Wasąg P, Zakrzewski P, Lenartowska M, and Lenartowski R. 2017. Calreticulin is required for calcium homeostasis and proper pollen tube tip growth in *Petunia*. *Planta* 245: 909–926.

Symbiotic associates of treehoppers (Hemiptera: Cicadomorpha: Membracidae): ultrastructure, distribution and transovarial transmission

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Treehoppers, like other plant sap-sucking hemipterans, are host to symbiotic microorganisms which supplement their restricted diet with amino acids (Baumann, 2005). Microscopic observations revealed that in the abdomen of treehoppers *Centrotus cornutus*, *Gargara genistae* and *Stictocephala bisonia* large organs termed bacteriomes are present. Bacteriomes are composed of giant bacteriocytes which are tigthly packed with symbiotic bacteria. Molecular analyses revealed that *S. bisonia* harbors two bacterial symbionts of ancient origin: *Sulcia* (Bacteroidetes) and *Nasuia* (Proteobacteria: Betaproteobacteria). *C. cornutus* and *G. genistae* apart from ancestral symbionts harbor gammaproteobacterial associates of more recent origin: bacterium *Arsenophonus* and bacterium *Serratia* (respectively). Additionally, in *C. cornutus* and *G. genistae* bacteria *Rickettsia* are present. In all the examined treehopper species bacteria *Sulcia* and *Nasuia* are localized in the own bacteriocytes whereas *Arsenophonus* and *Serratia* occur both in the own bacteriocytes as well as in bacteriocytes with bacteria *Nasuia*. Bacteria *Arsenophonus* and bacteria *Serratia* co-residing with *Nasuia* undergo autophagic degradation. Microscopic observations revealed that all the symbiotic bacteria in *C. cornutus*, *G. genistae* and *S. bisonia* are transovarially transmitted from the mother to the progeny. After leaving the bacteriocytes symbionts start to invade the posterior ends of ovarioles containing terminal oocytes at the stage of the advanced vitellogenesis. Microorganisms migrate to the perivitelline space through the cytoplasm of follicular cells surrounding the posterior pole of the oocyte. After passing through the fillicular epithelium symbionts gather in the deep invagination of oolemma.

Obtained results indicate that: (1) *S. bisonia* retainded the ancestral symbiotic system: "*Sulcia* and *Nasuia*", (2) the symbiosis in *C. cornutus* and *G. genistae* represents the transitional state in which the novel symbionts *Arsenophonus* and *Serratia* began already to replace the bacterium *Nasuia*.

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REFERENCES

Baumann P. 2005. Biology of bacteriocyte-associated endosymbionts of plant sup-sucking insects. *Annual Review of Microbiology* 59: 155–189.

Physiological aspects of sexual reproductive potential of *Stevia rebaudiana* Bertoni cultivated in moderate climate conditions

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Aside the genetic control that integrates endogenous and environmental cues, the transition from vegetative to generative state of plants is equally controlled by phytohormones, by gibberellins (GAs) mostly. In angiosperms, GAs promote development of male and female floral organs, their signaling regulates processes in pollen, in anther tissue as well as in pistil and ovule (Plackett and Wilson, 2016). Successful fertilization, viable seed and fruit development is a key regulatory function of GAs.

Stevia rebaudiana Bertoni, is the perennial plant, sweet herb of Paraquay (Ramesh et al., 2006). The leaves owe their sweetness to diterpene compounds – steviol glycosides (SVglys) which have no caloric value. Thanks to many valuable properties used in pharmacy and medicine, stevia is now cultivated in many places all over the world. However, introduction of this species in agricultural production is limited due to plants propagation since seed germination is very poor and seedlings are very difficult to achieve.

Our studies were carried on stevia AX strain, described as the only one capable to proceed all developmental stages in moderate climate conditions (Libik-Konieczny et al., 2018). Results revealed the proper way of male and female gametophyte development typical for Asteraceae family and high viability of pollen grains. However, some problems with pollen germination and subsequent seed formation were observed. Thus we suppose that it could be caused by the noted insufficient level of GAs during flowering caused in terms with the increasement of SVglys formation which share some common points in their biosynthetic pathway with GAs biosynthesis (Brandle and Telmer, 2007).

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REFERENCES

Brandle JF, and Telmer PG. 2007. *Phytochemistry* 68: 1855–1863.

Libik-Konieczny M, Capecka E, Kąkol E, Dziurka M, Grabowska-Joachimiak A, Śliwińska E, and Pistelli L. 2018. *Scientia Horticulturae* 234: 10–18.

Plackett ARG, and Wilson ZA. 2016. *Annual Plant Review* 49: 323–358.

Ramesh K, Singh V, and Megeji NW. 2006. *Advances in Agronomy* 86: 137–177.

Origin, development and functioning of serial abdominal outgrowths in a viviparous earwig, *Arixenia esau* (Dermaptera, Arixeniidae)

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The embryos and first instar larvae of the epizoic earwig, *Arixenia esau* develop sequentially in two different compartments of the female reproductive system, *i.e.* ovarian follicles and the uterus (Tworzydlo et al., 2013). Our analyses revealed that abdominal segments of the advanced embryos and larvae, growing inside the uterus, are equipped with paired serial multilobed outgrowths. The outgrowths bud from the lateral parts of the abdominal nota and persist until the end of intrauterine development. The lobes of the outgrowths adhere to the epithelium lining the uterus and form a series of small contact sites. We suggest that these contact sites collectively constitute a dispersed placenta-like organ involved in the nourishment of the embryo. We also show that the bundles of muscle fibers associated with the abdominal outgrowths facilitate flow of the haemolymph from the lumen of the outgrowths to the larval body cavity. Following the completion of the intrauterine development, abdominal outgrowths are shed with larval cuticle during the first molt. Using immunohistochemical and biochemical approaches, we demonstrate that the *Arixenia* abdominal outgrowths represent an evolutionary novelty related to viviparity and intrauterine development, and suggest that they are not related to wing homologs (Clark-Hachtel and Tomoyasu, 2016).

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REFERENCES

Tworzydlo W, Kisiel E, and Bilinski SM. 2013. Embryos of the viviparous dermapteran, *Arixenia esau* develop sequentially in two compartments: terminal ovarian follicles and the uterus. *PLoS One* 8: e64087.

Clark-Hachtel CM, and Tomoyasu Y. 2016. Exploring the origin of insect wings from an evo-devo perspective. *Current Opinion in Insect Science* 13: 77–85.

Evolution of flower structure and pollination strategy in *Viola* L.: from 'nectar flowers' to 'pollen flowers'

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The genus *Viola* L. with over 600 species grouped into 17 sections, distributed on both hemispheres, developed cleistogamous flowers (CL) adapted to self-pollination and chasmogamous flowers (CH) to cross-pollination by insects. In floral evolution, 'nectar flowers' with anther appendages (nectaries) producing nectar are considered to be a primitive state contrary to 'pollen flowers' with reduced spur and anther appendages, scanty nectar secretion treated as derived traits (Freitas and Sazima, 2003). To analyze the evolutionary trend, we examined flower structure of species from different *Viola* sections using light, transmission and scanning electron microscopy, and solid-phase microextraction followed by gas chromatography and mass spectrometry (SPME–GC/MS) analysis for the flower scent composition. Flowers of *Chamaemelanium*, *Melanium*, *Nosphinium*, *Plagiostigma* and *Viola* sections have traits characteristic for 'nectar flowers'. Non-cleistogamous *Viola banksii* of Australian, endemic *Erpetion* sect. develops spurless CH flowers, anther glands not functioning as nectaries, night-closing petals facilitating self-pollination (Kwiatkowska et al., 2019). As relatively young section (ca. 5 million-year-old, Marcussen et al., 2015) it develops derived traits, reflecting adaptations for self-pollination in the absence of insect pollinators. The delicate scent of CH flowers and petal venation patterns suggested adaptation to insect pollination. Although the primary function of floral scent is to attract pollinators, volatile chemicals have also additional functions, including defense and protection against abiotic stresses (Knudsen et al., 2006).

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REFERENCES

Freitas L, and Sazima M. 2003. *Annals of Botany* 91: 311–317.

Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. 2006. *The Botanical Review* 72: 1–120.

Kwiatkowska M, Bohdanowicz J, Cubała M et al. 2019. *Flora* 253: 1–9.

Marcussen T, Heier L, Brysting AK et al. 2015. *Systematic Biology* 64: 84–101.

POSTERS

Temperature and oxygen during development cause common rough woodlice (*Porcellio scaber*) to alter the size of their gas-exchange organs

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Terrestrial isopods have evolved pleopodal lungs that provide access to the rich aerial supply of oxygen. However, isopods occupy conditions with unpredictable thermal and oxygen gradients (Wright and Ting, 2006), suggesting that they might have evolved adaptive developmental plasticity in their respiratory organs to meet metabolic demand in hypoxic conditions.

To explore this, we conducted an experiment in which we reared common rough woodlice (*Porcellio scaber*) from eggs to maturation at different temperatures (15 and 22°C) and different oxygen levels (10% and 22% O_2). We sampled developing females and mature (both sexes) animals. We compared the area of their pleopod exopodites (proxy of lung size) and the shape of von Bertalanffy's (von Bertalanffy, 1957) growth curves.

Woodlice grew relatively faster but achieved decreased asymptotic body mass in response to warm conditions but not to the oxygen level. In hypoxia, growing females developed larger lungs compared to normoxia, but only in the late stage of development. This effect was present also in mature males. Woodlice reared in warm had smaller lungs (the effect increased with developmental time).

Our results demonstrated that woodlice exhibit phenotypic plasticity in lung size which helps them equilibrate gas exchange capacity to differences in the oxygen supply and metabolic demand along environmental gradients. The complex pattern of plasticity might indicate the effects of a balance between water conservation and oxygen uptake, which would be especially pronounced in mature females that need to generate an aqueous environment inside brood pouch.

REFERENCES

Von Bertalanffy L. 1957. Quantitative Laws in Metabolism and Growth. *The Quarterly Review of Biology* 32: 217–31.

Wright JC, and Ting K. 2006. Respiratory Physiology of the Oniscidea: Aerobic Capacity and the Significance of Pleopodal Lungs. *Comparative Biochemistry and Physiology, Part A* 145: 235–44.

Ploidy impact on the functioning of natural *Carassius gibelio* polyploids (Pisces, Teleostei)

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Our last findings reveal a higher incidence of sexually-reproducing *C. gibelio* diploids ($2n = 100$) of both sexes, which may have facilitated their success in invading Europe (Przybył et al., 2020). The occurrence of *C. gibelio* 2n and 3n females (2nF, 3nF) and males (2nM, 3nM) in the Siemianówka Reservoir inspired us to compare the impact of ploidy on: body length and weight; the levels of testosterone (T), 11-ketotestosterone (11-KT) and 17-β estradiol (E_2) in the plasma and gonads (ELISA) as well as the gonadal development (by histology) and gonadosomatic index (GSI) before, during and after spawning; oocytes and eggs size, and absolute fecundity (Fa).

The weight-length relationships were related to sex, but not to the ploidy. Nested analysis of variance indicated that the concentration of T and 11-KT described by PC-1 depends on the sex, season and ploidy of individuals, but the level of these hormones did not differ between 2nF and 3nF regardless of the period of the breeding season. Much higher concentration of T and 11-KT in males than in females was not affected by their ploidy. The concentration of E_2 described by PC-2 did not depend on the ploidy, but on the period of the breeding season and the fish sex. Histologically the ovaries and testes revealed no differences in 2nF-3nF and 2nM-3nM, respectively. In the case of females, GSI was significantly affected by the period of the spawning season and the ploidy ($p < 0.05$). 3nF characterized by larger oocytes in vitellogenic stages and eggs compared to 2nF. Fa of 3nF was statistically significantly ($p < 0.05$) lower than the Fa of 2nF. The results belong to the few concerning naturally occurring individuals of the same species with different ploidy inhabiting the same population.

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REFERENCES

Przybył A, Przybylski M, Spóz A, Juchno D, Szabelska A, Kowalewska K, and Boroń A. 2020. Sex, size and ploidy ratios of *Carassius gibelio* from Poland. *Aquatic Invasions* 15 (in press).

Formation of cell lines in tetraploid mouse blastocyst

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Although it is known that preimplantation tetraploid embryos have such abnormalities as greater cell volume, half of the normal cell number, delay in cell cycle and changed expression of multiple genes (Tarkowski et al., 1977; Park at al., 2011; Kawaguchi et al., 2009), it is unclear how cell lines of blastocyst are formed in them.

Tetraploid mouse embryos are commonly used in the tetraploid complementation to rescue embryonic lethality or generate mice from embryonic stem cells. Due to this procedures, knowledge of tetraploid blastocyst development should be extended. Here we examined formation of blastocyst cell lines (trophectoderm, epiblast and primordial endoderm) in tetraploid embryos produced by the fusion of blastomeres from two-cell diploid embryos in electric field. That was compared to development of cell lines in diploid embryos which have not been under electric field impact and with diploid embryos which have been exposed to the electric field but did not fused.

This experiment proved that formation of cell lines in diploid and tetraploid blastocysts proceeds differently. We observed that the cell number in tetraploid embryos was lower than half of the cell number of diploid embryos which have not been exposed to the electric field, cell cycles of their blastomeres were delayed and due to the delay of trophectoderm lineage development the ratio of number of inner cell mass cells to trophectoderm cells was higher in tetraploids. However, we demonstrated that these differences were not the consequence of tetraploidy, but resulted from the exposition of embryos to the electric field.

REFERENCES

Tarkowski AK, Witkowska A, and Opas J. 1977. Development of cytochalasin in B-induced tetraploid and diploid/tetraploid mosaic mouse embryos. *Journal of Embryology and Experimental Morphology* 41: 47–64.

Park MR, Lee AR, Bui HT, Park C, Park KK, Cho SG, Song H, Kim JH, Nguyen VT, and Kim JH. 2011. Chromosome remodeling and differentiation of tetraploid embryos during preimplantation development. *Developmental Dynamics: An Official Publication of the American Association of Anatomists* 240: 1660–69.

Kawaguchi J, Kano K, and Naito K. 2009. Expression profiling of tetraploid mouse embryos in the developmental stages using a cDNA microarray analysis. *The Journal of Reproduction and Development* 55: 670–75.

The impact of methoxychlor neonatal exposure on cell proliferation in neonatal and adult pig uteri

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Methoxychlor (MXC) is an organochlorine pesticide, used worldwide till early 2000s. MXC was banned for its toxicity, bioaccumulation, and endocrine disruption activity. This pesticide with estrogenic/antiestrogenic/ antiandrogenic properties alters the reproductive function and behaviour. The exposure to MXC during the fetal and neonatal periods causes dysfunction in reproductive tract that extend into adulthood. The observed changes concern acceleration of vaginal competency, onset of estrus, irregular estrous cycles, prolonged estrus, low pregnancy rate, small litter size and early reproductive maturity.

The aim of the study was to examine the effects of exposition to methoxychlor during neonatal period, on uterine morphogenesis and cell proliferation in neonatal and adult pigs' uteri. Neonatal pigs were injected with methoxychlor (MXC-20μg/kg bw) between days 1 to 10 *post partum* (n=8 for each group) or corn oil (control, n=8 for each group). The uteri were collected in the experimental groups from 11-day-old pigs (n=4) or adult pigs (n=4) in their second estrous cycle. Part of uterus from each animal was fixed for immunohistochemistry (IHC) and the other was frozen and used for Western blot analysis (WB) as well as quantitative Real-Time PCR.

In uteri obtained from neonatal pigs no significant differences were observed in endometrium thickness and number of uterine glands. Although, in adult uteri increased number of small arteries and veins with thicker walls were observed. In neonatal uteri the MXC treatments resulted in a significantly lower protein abundance of PCNA compared to the control group, whereas in adult uteri no significant differences were observed.

Obtained data show that methoxychlor treatment in neonatal period may cause changes in uterine morphology in both neonatal and adult pigs' uteri and cell proliferation in neonatal pigs.

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Mitotic abnormalities observed in gonocytes of *Pelophylax esculentus* linked to hybridogenesis

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The basic principle of hybridogenesis is a specific modification of meiosis, which enables formation of functional gametes in certain interspecies hybrids (e.g. *Bacillus rossius-grandii benazzii* – stick insects, fish of the genus *Poeciliopsis*, water frogs *Pelophylax esculentus*). This modification is possible due to the unification of the genomic composition of germ cells by eliminating entire genome of one of the parental species. The second non-recombinant genome remains in the gametes.

Despite the knowledge of organisms that persist through hybridogenesis, cellular and molecular processes leading to genome elimination are not fully understood. Although it was established that genome elimination occurs via formation of micronuclei (Chmielewska et. al., 2018), it is possible that mitosis also takes part in it. Our research shows that in *Pelophylax esculentus* hybridogenesis is exceptionally complex and does not follow all general rules, because a distinct group of germ cells shows variable genomic compositions. Moreover, we recorded the alterations of mitotic processes in gonocytes (oogonia and spermatogonia) from tadpoles: multipolar mitoses, premature sister chromatid separation, misaligned and lagging chromosomes. This may lead to polyploidy and aneuploidy, which we confirmed in gonocytes, spermatogonial stem cells, spermatocytes and spermatozoa using the cytogenetic techniques. Additionally, lagging chromosomes may result in micronuclei formation, being the second mechanism of genome elimination apart from the chromatin budding during interphase.

Similar morphological phenomena have been observed in cancer cells in which the underlying molecular processes have already been studied. Mitotic aberrations can be caused by an incorrectly functioning M-phase checkpoint, whose overactivity causes lagging chromosomes or cell cycle reversal to S-phase, and its weakness – premature separation of individual chromosomes. Each of them could lead to further aberrations in interphase or to the programmed cell death and necrosis.

In the course of further research, we aim to confirm that the mitotic abnormalities during hybridogenesis are similar to those appearing in tumorigenesis, and to find out possible molecular pathways.

REFERENCES

Chmielewska M, Dedukh D, Haczkiewicz K, Rozenblut-Kościsty B, Kaźmierczak M, Kolenda K, Serwa E, Pietras-Lebioda A, Krasikova A, and Ogielska M. 2018. The programmed DNA elimination and formation of micronuclei in germ line cells of the natural hybridogenetic water frog *Pelophylax esculentus*. *Scientific Reports* 8: 7870.

Morphology of the olfactory organs of three demersal species of sharks – comparative study (preliminary reports)

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Among Chondrichthyes there is a diversity of structure of olfactory organ. In neoselachians (modern sharks and rays) a nasal cavity placed between an anterior and posterior nostril is filled with olfactory lamellae with secondary folds (reviewed in Cox, 2013).

The olfactory lamellae are lined with olfactory epithelium consisted of sensory component found within most of the lamella and nonsensory one covering the remaining surface. Literature data shows that, in opposite to teleosts, sensory epithelium of neoselachians is composed of dominating microvillus OSNs and very rare crypt OSNs, whereas there is lack of ciliated OSNs. Nonsensory epithelium is formed by ciliated cells and rare goblet cells (Theisen et al, 1986).

The aim of our study was to analyze structure and ultrastructure of olfactory organ of demersal sharks, etmopterid *Etmopterus spinax* and scyliorhinids *Scyliorhinus canicula* and *Galeus melastomus* in comparative aspect. All studied species possess microvillus OSNs in sensory component (SEM). Within nonsensory compartment dominate ciliated cells, but the species are various in terms of number and arrangement of goblet cells. Moreover, these species differ in the development of secondary lamellae surfaces. Furthermore, in *S. canicula* giant cells and ciliated OSNs with short cilia placed between the epithelial cells were observed in SEM – the first report. However, it is necessary to undertake additional tests in TEM and confocal microscopy using immunohistochemical methods to correctly identify them.

REFERENCES

Cox J. 2013. Ciliary function Diversity and Natural History. *Fish and fisheries* 14: 364–390.

Theisen B, Zeiske E, and Breucker H. (1986) Functional morphology of the olfactory organs in the spiny dogfish (*Squalus acanthias* L.) and the small-spotted catshark (*Scyliorhinus canicula* L.). *Acta Zoologica* 67: 73–86.

Embryonic development of the nematode *Cystidicola farionis* at different temperatures

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A highly pathogenic nematode *Cystidicola farionis* is a common parasite of salmonid fish located in the swim bladder. Its prevalence depends on the fish age, the feeding strategy and the season of the year. Amphipoda crustaceans are intermediate host of this nematode. Since younger fish usually prey in the profundic and pelagic zones, where these crustaceans do not occur, the intensity of infestation in younger fish is usually much lower than in older ones. An increased mortality of fish as a result of infection with the nematode was especially observed in autumn, when amphipods (intermediate host) availability as a food item for fish was increased.

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 Cystidicolidae (*C. farionis)*) are classified as "thin-shells" eggs, and during an embryonic development they display a high susceptibility to abiotic factors as temperature and water oxygenation.

The present study was aimed at following temperature effects on the embryonic development of the parasite. Adult nematodes were dissected from the swim bladder smelt *Osmerus eperlanus* caught in the Vistula Lagoon. Using a dissection needle, eggs were isolated from the terminal part of the uterus of females and suspension into 3 parts and were placed at different temperatures (4°C, 15°C and 23°C). Egg development in all the samples was checked daily under a Biolar compound microscope at 20 x 12,5 magnification.

The eggs of *C. farionis*, isolated from the uterus are oval with polar filaments and surrounded by a double, transparent proteinaceous membrane. No egg development was observed on the culture start; about 2% of the eggs showed blastomere stage 2 only. Development was asynchronous and temperature dependent. On day 2, all the eggs in the sample kept at 15°C showed the blastula stage. On day 3, the late gastrula stage was recorded, whereas on day 4 most of the eggs contained larvae. The eggs kept at 4°C and 23°C developed at a much slower rate; the developmental stages appeared about 5 days later, compared to the sample.

The lingual papillae of the tongue of the neonate common hippopotamus *Hippopotamus amphibius* (Artiodactyla, Hippopotamidae)

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The common hippopotamus (*Hippopotamus amphibius)* is one of two members of the Hippopotamidae family that are enlisted in the IUCN Red List of Threatened Species as a vulnerable species. The microstructure of the tongue has been described in an adult *Hippopotamus amphibious* (Yoshimura et al., 2009) and a neonate *Choreopsis liberiensis* (Goździewska-Harłajczuk, 2019). However, there is no analysis of the lingual papillae in the neonate common hippopotamus, thus the aim of the study was to describe the macrostructure of the superficial lingual papillae in this neonate animal. The tongue was obtained from one common hippopotamus from the Wrocław Zoo. The animal died naturally. According to the presiding law in the European Union, there is no need for consent from the local Ethical Committee for the collection of samples post-mortem. The tongue was 21 cm long. The surface of the tongue was not pigmented. The dorsal surface of the tongue was covered by mechanical papillae: most numerous filiform papillae on the apex and body of the tongue and conical papillae covering the root of the tongue. The second group of papillae were the gustatory papillae including the fungiform papillae and foliate papillae on the caudo-lateral surface of the tongue. There were 4–8 fungiform papillae/cm^2 on the apex and body of the tongue. The foliate papillae were formed from 12–14 slit-like parallel grooves. There were no marginal papillae, which have been reported in some neonate and young mammalian species. The lingual papillae of the neonate common hippopotamus showed similarity to the lingual papillae of the tongue of the pygmy hippopotamus. The lingual prominence was more developed in the adult common hippopotamus.

REFERENCES

Goździewska-Harłajczuk K. 2018. Histological study of the tongue of the neonate Pygmy hippopotami (Choreopsis liberiensis, Cetartiodactyla: Hippopotamidae). Acta Biologica Cracoviensia Series Botanica, 60: suppl 1, 55.

Yoshimura K, Hama N, Shindo J, Kobayashi K, And Kageyama I. 2009. Light and scanning electron microscopic study on the tongue and lingual papillae of the common hippopotamus, Hippopotamus amphibius amphibius. Anatomical Record 292: 921–934.

The influence of GFP transgene expression on cell function in *Drosophila* maturing spermatids

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Green fluorescent protein (GFP) is a valuable tag to monitor complex cellular processes. It is commonly believed that expression of GFP-tagged transgene does not influence endogenous gene expression and does not alter cell physiology. However, several lines of evidence indicate that GFP can induce side effects that can influence proper functioning of cells or animals. Even GFP transgenes are commonly used by scientists, they rarely perform control experiments to assess whether or not cells or organisms expressing GFP differ in structure and function from wild type (WT). If such controls are not performed, results might be misinterpreted and experimental artefacts might be ignored.

Several different GFP transgenes have been used in studies examining the role of myosin VI (MYO6) in *Drosophila* spermiogenesis, called spermatid individualization (Noguchi et al., 2009; Isaji et al., 2011). Males expressing full-length GFP-MYO6 are used as the positive control in these studies, also by us. Therefore, the aim of the present work was comparative analysis of maturing spermatids of WT, GFP-MYO6EG/EN (express full-length exogenous GFP-MYO6 transgene in the presence of endogenous MYO6), GFP-MYO6EG (express GPP-MYO in the absence of endogenous MYO6), and MYO6-deficient (*jaguar* mutant) *Drosophila* males. Our cytochemical and immunocytochemical studies using confocal microscopy and transmission electron microscopy show that expression of a transgene GFP-MVI_{EG} causes subtle ultrastructural changes in *Drosophila* maturing spermatids when expression of endogenous MYO6 is silent but does not affect male fertility. From these data we conclude that, although expression of GFP-MVI_{EG} does not impair spermatogenesis and production of functioning sperm, it does slightly alter the normal physiological process of sperm individualization.

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REFERENCES

Noguchi T, Frank DJ, Isaji M, and Miller KG. 2009. Coiled-coil-mediated dimerization is not required for myosin VI to stabilize actin during spermatid individualization in *Drosophila melanogaster*. *Molecular Biology of the Cell* 20: 358–367.

Isaji M, Lenartowska M, Noguchi T, Frank DJ, and Miller KG. 2011. Myosin VI regulates actin structure specialization through conserved cargo-binding domain site. *PLoS ONE* 6: e22755.

Further studies on interracial hybrids in *Rumex hastatulus*

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Rumex hastatulus is an endemic dioecious American species with two chromosomal races: Texas $2n=10$ (XX/XY) and North Carolina $2n=8$, 9 (XX/XY1Y2). Previous studies on the hybrids derived from experimental reciprocal crossing between these races revealed decreased male fertility and rarity in the T×NC hybrid, thus the evidence of Haldane's rule (HR) in this species was demonstrated (Kasjaniuk et al., 2019). This is the first case of plant in which the full expression of HR was found. It was argued that the cause of reduced hybrid fertility is the production of genetically unbalanced pollen grains, but it was confirmed only indirectly (by comparison of aceto-carmine staining and expected frequency of pollen genotypes). The reason of male rarity remained unknown. Therefore, detailed research has been undertaken on these issues using flow-cytometric measurements of pollen DNA and analysis of photosynthetic apparatus and antioxidant capacity of hybrids and the parental forms. It was shown that the differences in the nuclear DNA amount make it possible to distinguish male- (M) and female-determining (F) pollen grains in all cytotypes. The average M : F ratios were balanced in NC and NC×T, slightly male biased in T and heavily biased in T×NC. In the latter hybrid only two of the four expected pollen genotypes were evidenced. It suggests that M : F ratio among its progeny should be prezygotically determined. Physiological analyses showed differences in photosynthetic capacity and hydrogen peroxide content in males and females of analysed forms, and that the T×NC hybrid differs in this respect from the others. This correlated with the activity of some enzymatic antioxidants that are among others responsible for maintenance of redox homeostasis.

REFERENCES

Kasjaniuk M, Grabowska-Joachimiak A, and Joachimiak AJ. 2019. Testing the translocation hypothesis and Haldane's rule in *Rumex hastatulus*. *Protoplasma* 256: 237–247.

Melatonin secretion form the pineal organs of goose embryos *in vitro*

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The synthesis of melatonin (MLT) begins in the avian pineal organ during the period of embryonic life and occurs in a diurnal rhythm when eggs are incubated in a light-dark cycle. However, our knowledge on this process derives almost exclusively from the studies performed in one species, the domestic chicken. The aim of this study was to characterize secretion of MLT from the embryonic pineal organs of the domestic goose *in vitro*. The glands were removed from eggs incubated in 12L:12D cycle on days 18, 20, 22, 24, 26, and 28 of the embryonic life (ED 18–28) and placed in superfusion culture for 4 days. The pineal organs of all investigated groups of embryos released MLT, however the level of secretion increased prominently with the age of embryos. The glands of embryos taken on ED 18 and 20 showed no night-time increase in MLT secretion during the first day of incubation in 12:12D cycle, but they demonstrated the prominent diurnal changes in secretion on the next three days of culture. The MLT secretion increased stepwise during scotophase and decreased during photophase. The night-time increase in MLT secretion was very small on the first day of culture in the pineal organs of embryos taken on ED 22 and prominent in those taken on ED 26–28. Interestingly, the level and amplitude of diurnal variation in MLT secretion increased markedly in a course of culture in all age-groups. During incubation in the reversed cycle, the glands entrained their secretory activity to new light condition starting from the second day of culture. The endogenously-generated circadian changes in MLT secretion were observed only during the first day of culture in continuous darkness and exclusively in the glands taken on ED 24–28. In conclusion, the pineal organs of goose embryos secrete MLT as early as on ED 18 and express the light-controlled diurnal rhythm of MLT secretion during the first day of culture starting from ED 22. The artificial conditions of culture influence the secretory activity of embryonic pineal organs.

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A comparative study of male germ cells ratio in the gonads of *Cobitis taenia* (Teleostei, Cobitidae) from diploid and diploid-polyploid populations

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Cobitis taxa are small-sized, bottom-dwelling fishes indigenous to Europe and Asia. They exist in exclusively diploid bisexual populations consisting of spined loach, *Cobitis taenia* Linnaeus, 1758, but mostly they occur in mixed diploid-polyploid (d-p) hybrid populations where *C. taenia* co-exist with hybrid triploid asexual females and hybrid tetraploids of both sexes. The aim of the study was to examine the proportion of the different cell types in the testes of *C. taenia* from diploid as well as d-p population before, during and after spawning.

The study was carried out on 12 males of diploid *C. taenia* from an exclusively diploid population in Legińskie Lake and 12 males of diploid *C. taenia* from mixed, d-p population in Pilica River (Poland). From each group, four fishes were collected in pre-spawning, spawning and post-spawning period. Testes were fixed in 10% formalin in phosphate buffered saline, dehydrated and then paraffin-embedded. Sections of 5 μm thick were stained with hematoxylin and eosin. To calculate the proportion (%) of testicular germ cells (spermatogonia, spermatocytes, spermatids, spermatozoa), three randomly chosen digital fields (×200 image magnification, 825-points grid) were analyzed using ImageJ software.

Before spawning, the significantly higher number of spermatocytes ($p < 0.05$; 49±2.44%) and spermatids ($p < 0.05$; 5±0.77%) as well as lower number of spermatozoa ($p < 0.05$; 29±3.54%) was observed in testes of *C. taenia* from d-p population in comparison to *C. taenia* from diploid population (19±1.85%, 3±0.74%, 61±2.39%, respectively). Moreover, the higher amount of spermatogonia during spawning ($p < 0.05$; 16±2.32%) and after spawning ($p < 0.05$; 13±0.85%) was detected in *C. taenia* testes from d-p population than in males from diploid one (6±0.61% and 10±0.9%, respectively). Different ratio of particular germ cells in *C. taenia* from diploid and d-p populations indicated that those males exhibited no parallel development of spermatogenic cells and documented biological differences between this both populations.

Internal organization of the cysts in *Thulinius ruffoi* (Tardigrada, Isohypsibioidea: Doryphoribiidae)

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Tardigrades are worldwide occurring micrometazoans that can be found in varied environments. These microscopic animals developed various mechanisms as an adaptive strategy to withstand unfavorable environmental conditions. Cryptobiosis and diapause are seen as dormancy states in tardigrades. Encystment is a form of diapause and an ability to form cysts has been noted in many tardigrade species. Despite that, these findings are poorly documented and the number of studies on encystment in tardigrades is negligible. Cyst formation entails deep morphological changes. In a case of freshwater tardigrade *Thulinius ruffoi*, an animal forms a three-layered cuticular capsule and contracts its body. An individual closed inside the cuticular capsule is also covered with its own cuticle. The transparency of the cysts is significantly reduced and almost all organs are invisible when cysts are analyzed *in toto* using light microscopy (Janelt and Poprawa, 2020). In relation to encystment in tardigrades, histolysis has been suggested by some researchers (e.g. Murray, 1907; Kristensen unpublished data). Due to that, analysis of the internal organization of the cysts since their formation up to the six months was performed using transmission electron microscopy. Analysis of the cysts after a varied time of encystment duration showed that cells and tissues that form the internal organs are observed inside the body of the encysted individuals but as a consequence of its contraction are tightly packed. Elements of the muscular, nervous, digestive and reproductive systems were recognized. The oocytes nor eggs were never been observed inside the cysts of *Thulinius ruffoi*, however ultrastructural analysis showed that the ovary is occupied by the undifferentiated germ cell clusters. Moreover, free-floating storage cells and bacteria in the body cavity fluid were detected. In each, analyzed cyst the internal organs were observed even in individuals that were encysted for six months. Therefore, this research does not support the suggested connection between encystment and histolysis.

REFERENCES

Janelt K, and Poprawa I. 2020. Analysis of encystment, excystment, and cyst structure in freshwater eutardigrade *Thulinius ruffoi* (Tardigrada, Isohypsibioidea: Doryphoribiidae). *Diversity* 12, doi: 10.3390/d12020062.

Murray J. 1907. Encystment of Tardigrada. *Transactions of the Royal Society of Edinburgh* 45: 837–854.

Structure of the ovary in the pseudoscorpion *Cheiridium museorum* (Pseudoscorpionida, Cheiridiidae)

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Pseudoscorpions are matrotrophic chelicerates. The eggs of pseudoscorpions contain small amount of yolk. The embryos carried in the brood sac are nourished with the nutritive fluid synthesized in the female reproductive system (Weygoldt, 1969). It has been widely accepted that in pseudoscorpions the ovaries are bifunctional (Makioka, 1976). During the oogenic phase of the ovarian cycle the oocytes grow and undergo maturation, while in the secretory phase the ovaries are responsible for the nutritive fluid production. We examined the ovary structure during the oogenic phase in the pseudoscorpion *Cheiridium museorum*. Our studies show that in *Cheiridium* the ovary is an unpaired elongated organ of the exogenous (chelicerate) type. It means that growing oocytes protrude to hemocoel and maintain connection to the ovarian wall by means of epithelial cells that form oocyte stalks. Like in other pseudoscorpions the surface of growing oocytes is separated from hemocoel by follicular cells. However, compared to other studied so far pseudoscorpions, the ovary, during the oogenic phase, exhibits a distinct architecture. First, the female gonad contains a single germarium located in the middle part of the ovary. The germarium is occupied by oogonia and early previtellogenic oocytes accompanied by somatic interstitial and epithelial cells. Early previtellogenic oocytes bulge from the germarium, while oocytes in advanced previtellogenesis and vitellogenesis protrude from the ovarian wall. Second, the epithelial cells of the ovarian wall and the stalk cells have irregular shape and are equipped with long cellular processes. The latter observation suggests that the ovarian epithelial cells are migratory active.

REFERENCES

Makioka T. 1976. Alternative occurrence of two ovarian functions in the adult pseudoscorpion, *Garypus japonicus* Beier. *Acta Arachnologica* 27: 8–15.

Weygoldt P. 1969. *The Biology of Pseudoscorpions*. Harvard books in biology, no. 6. Harvard, University Press.

Expression of calnexin chaperone during pollen formation in *Petunia* anther

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Transmembrane protein calnexin (CNX) and its soluble homolog calreticulin (CRT) are related calcium (Ca^{2+})-binding proteins in the endoplasmic reticulum (ER) of eukaryotic cells. These lectin-like molecular chaperones constitute maturation system, known as the CNX/CRT cycle, which mainly promotes correct folding and oligomerization of newly synthesized glycoproteins before their export from the ER. Furthermore, both CRT and CNX play important role/s in Ca^{2+} homeostasis in animal and plant cells.

Pollen formation in angiosperms is a result of two sequential stages in the anther: development of haploid microspores during meiosis (microsporogenesis) and transformation of microsopores in mature pollen grains (microgametogenesis). Developmental process of the male gametophyte formation is associated with tissue/cell-specific expression of several genes that control cell division, chromatin remodeling, cytoskeleton functions, biogenesis of the sporoderm, and Ca^{2+}-dependent cell signaling pathway. We hypothesized that molecular chaperones such as CNX and CRT are expressed in developing anther, because a high rate of protein synthesis and intracellular Ca^{2+} homeostasis are strictly required during microsporo/gametogenesis. Indeed, we previously showed that *CRT* gene is highly expressed during pollen formation in *Petunia* anther (Wasąg et al., 2018). Here we show variable expression of the *CNX* gene during *Petunia* male gametophyte formation using fluorescent *in situ* hybridization (FISH) and species-specific molecular probe complementary to the *CNX* mRNA. We revealed spatiotemporal distributions of the *CNX* transcripts in functionally different anther tissues, including the male germline and the active tapetum. Based on our results we propose that CNX (probably together with CRT) is involved in molecular chaperoning during the key events of microsporogenesis within the anther.

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REFERENCES

Wasąg P, Suwińska A, Lenartowska M, and Lenartowski R. 2018. *Acta Biologica Cracoviensia, series Botanica* 60 suppl. 1: 80.

Reproductive ability of diploid and triploid *Carassius gibelio* (Pisces, Cyprinidae) females and males

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The gibel carp *Carassius gibelio* (Bloch, 1782) was introduced to Europe from China and the Amur River (Sakai et al., 2009) at the beginning of the 20th century and now is widely distributed across lakes and rivers. Until the 1990s, the majority of European *C. gibelio* populations consisted almost exclusively of triploid females reproducing by gynogenesis and represented discrete clonal lineages. Recently, in Poland, a relatively high number of mature males were documented, both diploid and triploid, indicating that some populations may be undergoing a transition of reproduction mode from unisexual gynogenetic to bisexual (Przybył et al., 2020). Mechanisms for the occurrence of variable proportion of males in natural populations are still unclear.

The purpose of the work was to check reproductive capacity of diploid and triploid *C. gibelio* females and males under controlled conditions. Two females and three males were caught before the breeding period from the diploid-triploid population in the Siemianówka Reservoir. Ploidy level was determined using a flow cytometry. In order to synchronize the maturity of females and males, the fish were subjected to hormonal injection. Diploid and triploid female were crossed with triploid and diploid male, respectively. Four days after fertilization, hatching occurred of all crosses. Some of the larvae obtained from the triploid female were characterized by deformations: mainly a C-shaped shortened body, pericardial hypertrophy and no eye pigmentation.

C. gibelio diploid and triploid males have been shown to be fertile and capable of inducing and/or fertilizing eggs derived from both diploid and triploid females. Obtained partial results require confirmation on a larger number of individuals.

REFERENCES

Przybył A, Boroń A, Przybylski M, Spóz A, Juchno D, Szabelska A, and Kowalewska K. 2020. Sex ratio and size of diploid and triploid *Carassius gibelio* – the most invasive freshwater fish species in Europe, from bisexual population in Poland. *Aquatic Invasions* 15.

Sakai H., Iguchi K., Yamazaki Y., Sideleva V.G., and Goto A. 2009. Morphological and mtDNA sequence studies on three crucian carps (*Carassius:* Cyprinidae) including a new stock from the Ob River system, Kazakhstan. *Journal of Fish Biology* 74(8): 1756–1773.

Protoplast fusion of selected accessions of *Brassica oleracea* var. *capitata* L. – effectiveness of the process and culture evaluation

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The experiments were conducted on three diploid accessions (cv. K1 and breeding lines: L1 and L2) of cabbage. Protoplasts were isolated from leaves of four-week-old plants cultured *in vitro* according to protocol of Kiełkowska and Adamus (2012, 2014). Fusion was performed in following combinations K1+L1 (FS1) and K1+ L2 (FS2). Controls (K1, K2, K3) were cultures of all accessions without fusion. Parental accessions were stained with fluorescein acetate (FDA) or rhodamine B (RB). Fusion was performed in multiporator (Eppendorf, DE). The overall efficiency of fusion (no. of fused protoplasts) was 8 % for FS1 and 13,5 for FS2. The efficiency of heterokaryons (recognized by double fluorescence FDA/RB) was lower and was 5,8 and 8,1% respectively for FS1 and FS2.

In order to track protoplast development, sterile cultures of fused cells and controls were established. After fusion protoplasts were immobilized in alginate layers and cultured in liquid medium (Kiełkowska and Adamus, 2012). Protoplast viability (FDA) day after isolation was high (approx. 90%) in FS1 and FS2 and dropped in the next five days on about few percentages. Mitotic activity of protoplast-derived cells in FS1 and FS2 was from 28 to 48% in fifth day of culture, while from 58 to 65% in fifteen day of culture. After approximately two months of culture calli development was observed. Calli clumps were freed from alginate layers and counted. Higher no. of calli (12–30/dish) was scored in controls. In cultures subjected to the fusion, on average 17 (FS2) and 21 (FS1) calluses/dish were observed. Colonies of calli were transferred to the solid regeneration medium. In all tested combinations shoot development was observed. Ploidy analyses (flow cytometry) of shoots from combinations subjected to the fusion shows that about 50% were tetraploids.

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REFERENCES

Kiełkowska a, and adamus a. 2012. An alginate layer technique for culture of *Brassica oleracea* L. protoplasts. *In Vitro Cellular and Developmental Biology – Plant* 48: 265–273.

Kiełkowska a, and adamus a. 2014. Embedding in filter sterilized alginate enhances *Brassica oleracea* L. protoplast culture. *Acta Biologica Cracoviensia Series Botanica* 56/2: 20–26.

Chondrogenic differentiation of human mesenchymal stromal cells derived from adipose tissue

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The term mesenchymal stromal cells (MSC) refers to multipotent progenitors with ability to differentiate into numerous types of cells. The high interest in MSCs is covered by the significant therapeutic potential of these cells. It has been proven that MSC can be isolated from almost every tissue of the human body. Adipose tissue has become the most promising MSC source due to easily and effectively obtainment with minimally invasive procedure. Immunomodulatory properties and high plasticity give ***A****dipose-****D****erived* ***M****esenchymal* ***S****tromal* ***C****ells* (**AD-MSC**) huge potential in regenerative medicine and tissue engineering (Bacakova et al., 2018; Louwen et al., 2018).

The aim of the study was to investigate differentiation AD-MSCs into chondrocytes. Material was collected from patient's white adipose tissue. In order to isolate AD-MSCs, adipose tissue was washed, minced, then undergone enzymatic digestion with collagenase and filtered through 70 μm and 40 μm strainer. AD-MSCs have been cultured *in vitro* on monolayer and examined using flow cytometry to confirm the MSC's phenotype. After third passage, induction of chondrogenesis began and lasted for two weeks. After that time AD-MSCs were fixed and stained.

The ability of the AD-MSCs to differentiate into chondrocytes has been assessed by histochemical staining using Toluidine Blue O solution. Chondrogenesis has also been detected by immunohistochemistry staining using anti-collagen type II antibody.

Staining confirmed AD-MSC's capability to differentiate *in vitro* into chondrocytes. The possibility of culturing and undergoing chondrogenesis of AD-MSCs on monolayer could be adopted in cell cultures on two-dimension scaffolds and forming engineered cartilage.

REFERENCES

Bacakova L, Zarubova J, Travnickova M, Musilkova J, Pajorova J, Slepicka P, Kasalkova NS, Svorcik V, Kolska Z, Motarjemi H, and Molitor M. 2018. Stem cells: their source, potency and use in regenerative therapies with focus on adipose-derived stem cells – a review. *Biotechnology Advances* 36: 1111–1126.

Louwen F, Ritter A, Kreis NN, and Yuan J. 2018. Insight into the development of obesity: functional alterations of adipose-derived mesenchymal stem cells. *Obesity Reviews* 19: 888–904.

Histological study of the upper and lower eyelids in the three neonate aardvark (*Orycteropus afer* Pallas, 1766) (Mammalia: Tubulidentata)

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In the present study, a histological analysis of the upper and lower eyelids in three females neonate aardvark (*Orycteropus afer* Pallas, 1766) was performed. The samples were collected from 2017 to 2019 from animals kept in the Wrocław Zoological Garden (Poland). The animals were not killed for the purpose of this study and were obtained post-mortem. The District Veterinary Officer authorised the collection of the study samples. According to the Polish and European law, studies on tissues obtained post-mortem do not require an approval of the Ethics Committee. The upper and lower eyelids were examined using light microscopy (H&E, picro-Mallory trichrome and Movat pentachrome (modified Russell Movat)). The upper and lower eyelids in the neonate aardvark contained an anterior surface covered by a cornified stratified squamous epithelium with 6 to 11 layers of nucleated cells. A thin lamina propria formed from loose connective tissue was located under the epithelium. Numerous sebaceous glands and sweat glands were located deep in the eyelash follicles in both eyelids. The eyelid stroma was formed from dense irregular connective tissue with a network of collagen and elastic fibers and nervous fibres. Moreover, there were numerous veins and arteries. The wall of the arteries was composed of the tunica media with smooth muscles cells and the tunica intima with internal elastic lamina. A thick layer of muscle that consisted of bundles of the orbicularis oculi muscle, levator anguli oculi medialis muscle and malaris muscle was observed in both eyelids. The superior tarsus of the upper eyelid and inferior tarsus of the lower eyelid consisted of dense fibrous connective tissue. No Meibomian glands were found in the study material. The posterior surface of both eyelids was covered by a stratified columnar epithelium with from 3 to 6 layers of cells with numerous goblet cells. No lymphoid follicles or diffuse lymphatic cells were found on the posterior surface of the upper and lower eyelids.

Cross-talk of uterine NK cells and trophoblast as determining factor of pregnancy success

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Pregnancy is a unique example of semi-allogeneic graft which is characterized by orchestrated changes in the immune system that occur between mother and fetus. A massive influx of leukocytes into the endometrium observed during the menstrual cycle in women creates the mild local inflammation milieu important for blastocyst implantation. The uterine NK (uNK) cells are the most abundant leukocytes at the maternal-fetal interface. Interestingly, data strongly suggest that uNK cells play a crucial role in remodeling of the maternal vessels that support placental development and function during pregnancy. The uNK cells express activating/inhibitory receptors that bind MHC class I molecules. Among various types of trophoblast only extravillous trophoblast cells express MHC class I molecule, HLA-C, HLA-E and HLA-G which are recognized by the uNK cells via KIRs, CD94–NKG2A/C and LILRB1 receptors, respectively (Parham, 2004). Proper uNK cell activation depends on interplay between maternal KIRs and fetal HLA-C. There are two main haplotypes of KIR receptors, KIR A and KIR B, that differ in the presence of many activating KIRs on B haplotype and only one generally non-functional activating KIRDS4 on A haplotype. HLA-C polymorphism manifests in the presence of more than 1000 alleles, which are divided in HLA-C1 and HLA-C2 group. HLA-C1 are recognized by the uNK cells via weak inhibitory KIR2DL2/3 receptors. HLA-C2 can be recognized by inhibitory KIR2DL1 and activating KIR2DS1 but stronger and more specific binding is observed between HLA-C2 and inhibitory KIR2DL1. Most frequently the combination of maternal AA KIR genotype and fetal C2 genotype leads to preeclampsia and fetal growth restriction (Colucci, 2017). Insufficient modulation of the uNK cell activity may also contribute to unexplained stillbirth, recurrent spontaneous abortion, primary infertility and failed in vitro fertilization (Moffet et al., 2016).

REFERENCES

Parham P. 2004. NK Cells and Trophoblasts: Partners in Pregnancy. *Journal of Experimental Medicine* 200: 951–955.

Colucci F. 2017. The role of KIR and HLA interactions in pregnancy complications. *Immunogenetics* 69: 557–565.

Moffett A, Chazara O, Colucci F, and Johnson Mh. 2016. *Reproductive BioMedicine Online* 33: 763–769.

Development of pancreas in lizard embryos – preliminary study

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Anlage of pancreas develops from different number of pancreatic buds. In general, pancreas develops as three pancreatic buds, one ventral and two dorsal. In some reptilian species pancreas develops from only two buds, because one of the dorsal bud degenerates. Development of the pancreas in lizard species is poor understood. Due to this fact the aim of this preliminary study was to investigate development of pancreas in chosen lizards: *Eublepharis macularius*, *Lepidodactylus lugubris* and *Anolis sagrei*. Embryos were isolated at regular intervals. Age of embryos was calculated according to specific developmental tables (Noro et al., 2009; Sanger et al., 2008; Wise et al., 2009). Pancreatic tissues were fixed in Bouin solution and stained with hematoxylin and eosine and Heidenhain's AZAN. Pancreas in studied species was first observed during the early developmental stage just after egg-laying. Initially, pancreas primordium was found in location similar to that in other vertebrate species, in the loop of duodenum, in close proximity to the liver anlage. At this stage pancreas was built from few ducts and small groups of cells then primordium started to elongate. At the end of embryonic life pancreas was formed by three protrusions. One of them was extended toward liver, second one was extended to spleen and third one was extended towards intestine. Results of this study revealed that location of pancreas anlage in *Eublepharis macularius* differed from that in *Lepidodactylus lugubris* and *Anolis sagrei* because in these two species one of pancreas protrusion was entirely surrounded by liver tissue. This situation was not observed in *Eublepharis macularius*.

REFERENCES

Noro M, Uejima A, Abe G, Manabe M, and Tamura K. 2009. Normal developmental stages of the Madagascar ground gecko *Paroedura pictus* with special reference to limb morphogenesis. *Developmental Dynamics* 238: 100–109.

Sanger TJ, Losos JB, and Gibson-Brown JJ. 2008. A developmental staging series for the lizard genus *Anolis*: a new system for the integration of evolution, development, and ecology. *Journal of Morphology* 269: 129–137.

Wise PA, Vickaryous MK, and Russell AP. 2009. An embryonic staging table for *in ovo* development of *Eublepharis macularius*, the leopard gecko. *Anatomical Record* 292: 1198–1212.

Myogenic regulatory factors during the grass snake (*Natrix natrix*) myogenesis

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The trunk muscle development is a multistep process involving mesodermal cells' determination and differentiation into adult muscles. Myogenesis in vertebrates is a developmental cascade governed by multiple factors. The crucial step for the entry of undifferentiated cells into the myogenic program is the induction of myogenic regulatory factors (MRFs). It is commonly known, that four genes are included in the regulatory genes family: *MyoD*, *Myf5*, *MRF4*, and *myogenin* (*MyoG*). Recent studies showed that *MyoD* and *Myf5* were expressed in muscle progenitor cells. On the other hand, *MRF4* and *MyoG* are activated in myoblasts and myotubes. Obtained results led to the conclusions, that *MyoD* and *Myf5* are responsible for the determination of myogenic progenitor cells, whereas *MRF4* and *MyoG* are required for the final determination of myoblasts (see Buckingham, 1994; Lewandowski et al., 2019).

The phenomenon of skeletal muscle formation at the morphological and molecular level was, excluding reptiles, well described in all vertebrates. Comparative analysis of myogenesis in the evolutionary context revealed gaps in the knowledge of MRFs expression among vertebrates (Lewandowski et al., 2019). The aim of our study was to fill the gaps concerning molecular factors involved in grass snake (*Natrix natix*) muscle differentiation. We also wanted to compare obtained data with results gathered from studies conducted on other vertebrates. Our aims were archived by analysis of subcellular localization and expression of four MRFs in selected developmental stages of reptile embryos. The analyses were carried out using a confocal microscope and Western blot method, respectively.

Our data allow us to conclude that besides strong similarities between myogenesis patterns occurring in different vertebrates, there are also some discrepancies.

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REFERENCES

Buckingham M. 1994. Muscle differentiation: which myogenic factors make muscle? *Current Biology* 4: 61–63.

Lewandowski D, Dubińska-magiera M, Migocka-patrzałek M, Niedbalska-tarnowska J, Haczkiewicz-leśniak K, Dzięgiel P, and Daczewska M. 2019. Everybody wants to move – evolutionary implications of trunk muscle differentiation in vertebrate species. *Seminars in Cell and Developmental Biology*, doi: 10.1006/j.semndb.2019.10.009.

May assisted reproductive technology (ART) cause the gametes and embryo epimutations?

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The gametes and embryonic manipulation methods, such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) are widely used in ART (Kohda, 2013). The novel laboratory techniques using in these methods improve the efficiency of ART. On the other hand, the dynamic changes in epigenetic profile of the embryo from its fertilization to implantation are well-known (Osman et al., 2018). The examinations of animals confirm that the various steps of the ART procedure may affect the epigenome gametes and embryos (Pinborg et al., 2016), however further research is needed to assess the incidence of this phenomenon in human (Osman et al., 2018). What's more, the procedure of ART should be improved to mimic the natural fertilization environment and avoid the epimutations. What is equally important, epimutations may be an effect of ART, but also epimutations may be a reason of infertility/ decreased fertility. There are much more questions. How effective are the embryo epimutations repair mechanisms? How effective are the repair mechanisms in embryonic epimutations? What is the role of placenta epimutations? How do epimutations influence baby and next generation health? Future research on human gametes and embryos manipulations is needed to assess the signification and consequences of imprinting errors (Osman et al., 2018).

REFERENCES

Kohda T. 2013. Effects of embryonic manipulation and epigenetics. *Journal of Human Genetics* 58: 416–420.

Osman E, Franasiak J, and Scott R. 2018. Oocyte and embryo manipulation and epigenetics. *Seminars in Reproductive Medicine* 36: e1-e9, doi: 10.1055/s-0039-1688801.

Pinborg A, Loft A, Romundstad LB, Wennerholm U-B, Söderström-Anttila V, Bergh C, and Aittomäki K. 2016. Epigenetics and assisted reproductive technologies. *Acta Obstetricia et Gynecologica Skandinavica* 95: 10–15.

Transovarial transmission of *Burkholderia* – the obligate symbiont in two scale insects from Eriococcidae family (Hemiptera, Coccomorpha)

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Obligate, mutualistic bacteria of plant sap-sucking hemipterans are usually maternally (vertically) inherited from one generation to the next. The modes of vertical transmission are different in particular hemipterans, however, in most of them, symbionts are transmitted transovarially, i.e. via female germ cells (Szklarzewicz and Michalik, 2017). We present the results of our microscopic and molecular investigations concerning bacterial symbionts in two scale insects belonging to Eriococcidae family: *Acanthococcus aceris* and *Gossyparia spuria*. These species are host to *Burkholderia* bacteria (Betaproteobacteria), which are localized in the cytoplasm of the fat body cells. According to our knowledge, the intracellular localization of *Burkholderia* bacteria has not been described before in any of the insects examined so far. An analysis of serial semithin sections has shown that *Burkholderia* bacteria infect ovarioles containing oocytes in the stage of late vitellogenesis. Bacteria invade the neck region of the ovariole. Symbionts migrate via cytoplasm of follicular cells or through spaces between them. Finally, symbionts accumulate in the perivitelline space (space between oolemma and follicular epithelium).

Metagenome sequencing has indicated that genomes of *Burkholderia* symbionts of *Acanthococcus aceris* and *Gossyparia spuria* are the smallest *Burkholderia* genomes yet sequenced. Their sizes are smaller than 900 kb and have a low GC content (~38%). Thus, during the evolutionary history of these symbionts a reduction of the genome occurred. In comparison with free-living *Burkholderia*, the symbiotic strains have fewer genes involved in housekeeping functions but retained genes responsible for biosynthesis of essential amino acids. This observation indicates that *Burkholderia* symbiont has a potential to play a similar role to other nutrient-supplementing insect symbionts.

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REFERENCES

Szklarzewicz T., and Michalik A. 2017. Transovarial transmission of symbionts in insects. In: Kloc M [ed.], *Results and Problems in Cell Differentiation,* 43–67. Vol. 63*, Oocytes. Maternal Information and Functions,* Springer, Cham.

The importance of pollen and seed characters in the phenetic taxonomy of the genus *Heliosperma* (Rchb.) Rchb. (*Sileneae*, Caryophyllaceae)

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Heliosperma (Rchb.) Rchb. (*Sileneae*, Caryophyllaceae) is recently reactivated monophyletic genus comprising 8–18 recognized species, almost all endemic to western Balkan countries. Diagnostic features of *Heliosperma* are conspicuous dorsal crest of long papillae on the seeds and petal limb segments laterally toothed or lobed (Frajman and Oxelman, 2007). The morphological differentiation into high elevation widely distributed taxa, and low elevation narrow endemics of *H. pusillum sensu lato* is not correlated with the molecular data and is a result of ecological differentiation (Trucchi et al., 2017). It is supported by studies of Niketić and Stevanović (2007) indicating that all species inhabiting low elevation crevices in gorges and canyons show a considerable morphological similarity contrary to *H. olivierae* resembling much more relic high mountain species, *H. macranthum*.

Micromorphological and anatomical characters are less influenced than the morphological by the environmental factors therefore we analyzed in LM and SEM microscopy pollen (3 characters) and seed (9 characters) of 6 taxa *sensu lato* of *Heliosperma* collected in the North of Albania. Pollen was uniform; spheroidal, pantoporate (more than 6-porate), did not differ in size between species and its viability was high (above 80%). Seeds microstructure was much more varied, seeds differed in *e.g.* number of row in crest, size, hilum position, etc.

In summary, seeds but not pollen characters are useful for species delimitation in the genus *Heliosperma*.

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This work was supported by the statutory funds of the Institute of Botany of the Jagiellonian University in Krakow (K/ZDS/005397).

REFERENCES

Frajman B, and Oxelman B. 2007. Reticulate phylogenetics and phytogeographical structure of *Heliosperma* (*Sileneae*, Caryophyllaceae) inferred from chloroplast and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 43: 140–155.

Niketić M, and Stevanović V. 2007. A new species of *Heliosperma* (Caryophyllaceae) from Serbia and Montenegro. *Botanical Journal of the Linnean Society* 154: 55–63.

Trucchi E, Frajman B, Haverkamp THA, Schönswetter P, and Paun O. 2017. Genomic analyses suggest parallel ecological divergence in *Heliosperma pusillum* (Caryophyllaceae). *New Phytologist* 216: 267–278.

Differences in embryo sac structure of various breeding lines of maize (*Zea mays* L.)

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Zea mays L. is an important crop and one of the model plants in embryology of angiosperms – especially in the investigation of double fertilization as well as embryo and endosperm development. Presence of three stages during egg cell development was earlier shown for a model genotype A188 (Mól et al., 2000). Here we attempt to confirm those steps for egg cell maturation in various unrelated genotypes of maize. Moreover, we followed the dimensions of the embryo sacs, the numbers of antipodals, and the presence of polar or secondary nuclei in the central cells. In total, 871 mature embryo sacs in 16 genotypes were analyzed in DIC optics after clearing of the ovule thick sections in methyl salicylate. In 8 genotypes all three egg cell stages were found. Two but the youngest stage were observed in the other 8 genotypes. This was possibly a result of various ear developmental rates in the field-growing plants. Variability in the embryo sack length (265±30 to 631±67 μm) or embryo sac width (120±6 to 165±13 μm) were found among genotypes. In 8 genotypes the length of the egg cell was not related to the embryo sac length. The number of antipodal cells varied from 17±4 to 58±12. In most of the analyzed central cells polar nuclei or fusing polar nuclei were found. In conclusion, despite of a reasonable variability of the embryo sac structure in various maize genotypes, the presence of three stages during maize egg cell differentiation has been confirmed.

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REFERENCES

Mól R, Idzikowska K, Dumas C, and Matthys-Rochon E. 2000. Late steps of egg cell differentiation are accelerated by pollination in *Zea mays*. *Planta* 210: 749–757.

Neb-colloostatin and its analogs, and conjugates of nanodiamonds with these peptides impair the metamorphosis of the *Tenebrio molitor* beetle

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The typical form of metamorphosis in beetles passes through four main stages: the egg, the larva, the pupa, and the imago and is called completed metamorphosis. The larva and the pupa, immature stages of beetle development are very different from the mature stage, the imago. The consequence of the undisturbed course of completed metamorphosis is that the insects reach sexual maturity, which results in the possibility of copulation and intensive reproduction. The reproductive success of harmful insects causes increased crop losses, or the spread of diseases caused by infectious pathogens, parasites or microbes, transmitted by insects into another living organism including human. For this reason, naturally occurring or synthetic molecules are sought that, by disturbing the metamorphosis of the pest, will limit its dispersion and reproduction.

In this study, we have shown that *Neb*-colloostatin, the pleiotropic insect peptide and its more potent hemocytotoxic analogues, interrupt metamorphosis of *Tenebrio molitor* beetle. The injection of the tested peptides caused serious morphological changes in larvae, pupae and the adult beetles that caused the lethal effects. Some of the adult individuals treated with these analogues had no wings and maintain larval body parts, e.g. legs. Nanodiamonds, regardless of a dose used, did not disturb the metamorphosis of *T. molitor*. However, the topical application of the conjugates consisting of nanodiamonds and the tested peptides disrupted metamorphosis of *T. molitor*. Malformations caused by the topical administration of the nanodiamonds and analogues complexes were similar to the pathological changes observed in development of the insect after injections of analogues.

This study demonstrated that *Neb*-colloostatin and its analogues, in addition to disrupting the *T. molitor* immune response and reproduction, significantly interfere with its development regardless of how they are introduced into the body of the insect.

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The insect ovary as a useful model for testing the gonadotropic activity of various molecules

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In the preliminary and application studies on an action of various molecules, it is crucial to understand the impact of them on reproduction and development of the embryo. The insect ovary is an useful model for the *in vivo* study of the gonadotropic activity of various compounds, including insect peptides, plant metabolites, enzymes, herbicides, or nanoparticles (Czarniewska et al., 2014; Ding et al., 2019; Lee et al., 2014; Zhang et al., 2018). A determination of: ovarian morphology, structure of ovarian follicles, presence of spaces between follicular cells, cell viability in germarium and vitellarium, size of terminal oocytes, number of eggs laid by females and hatching of larvae from eggs allows the identification of compounds that stimulate or inhibit activity of the insect' ovary or do not affect its function. The use of the insect' ovary in the short- and long-term study of the assessment of biological activity of various compounds allows the identification of natural or synthetic substances that in the future can be used as bioinsecticides.

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REFERENCES

Czarniewska E, Rosiński G, Gabała E, and Kuczer M. 2014. The natural insect peptide *Neb*-colloostatin induces ovarian atresia and apoptosis in the mealworm *Tenebrio molitor*. *BMC Developmental Biology* 14: 1–10.

Ding X, Huang X, Sun L, Wu J, and Liu J. 2019. Influence of abscisic acid-biosynthesis inhibitor fluridone on the feeding behavior and fecundity of *Nilaparvata lugens*. *Insects* 10: 1–12.

Lee S-H, Oh H-W, Fang Y, An S-B, Park D-S, Song H-H, Oh S-R, Kim S-Y, Kim S, Kim N, Raikhel A-S, Je Y-H, and Shin S-W. 2014. Identification of plant compounds that disrupt the insect juvenile hormone receptor complex. *Proceedings of the National Academy of Sciences of the United States of America* 112: 1733–1738.

Zhang J-L, Yuan X-B, Chen S-J, Chen H-H, Xu N, Xue W-H, Fu S-J, Zhang C-X, and Xu H-J. 2018. The histone deacetylase NIHDAC1 regulates both female and male fertility in the brown planthopper *Nilaparvata lugens*. *Open Biology* 8: 1–14.

The effect of high hydrostatic pressure on *in vitro* maturation, oocytes fertilization and development of pig embryos

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It was recently reported that high hydrostatic pressure (HHP) treatment of porcine zygotes enhances the developmental potential and quality of *in vitro* obtained blastocysts (Romek et al., Theriogenology, 2019). Therefore, the aim of the study was to determine the effect of HHP on *in vitro* maturation, fertilization of pig oocytes and development of embryos. The experiment was carried out on immature porcine oocytes obtained from gilt ovaries after slaughter. Immature oocytes assigned to HHP treatment (exp. group) were placed in a 0.5 mL droplet of manipulation medium and introduced into hot-welded straws. Next, the straws were put into pressure machine (Cryo-Innovation) for inducing HHP (38°C, press. 200 bar, 1h). After this time, the oocytes were matured to the Met II-stage in a modified TCM-199 medium (42–44 hours, 39°C, 5.0% CO_2). In the control group oocytes were matured without HHP treatment. After maturation, oocytes were denuded from cumulus cells and incubated with capacitated boar spermatozoa for 4 hours (39°C, 5.0% CO_2). After fertilization, putative zygotes were cultured *in vitro* in the NCSU-23 medium (39°C; 5.0% CO_2; 5.0% O_2) to the expanding blastocyst stage. A tendency to obtain a higher percentage of mature oocytes has been observed in the exp. group (93.1%) in which immature oocytes were exposed to HHP compared to the control group (90.7%), however the differences were not statistically significant. A slightly lower percentage of cleaved embryos (exp. group) was observed compared to the control group (37.8% and 46.7%, respectively) (the differences were not statistically significant). In contrast, a statistically significant ($p < 0.05$) lower morula percentage was found in the exp. group (39.3%) in which immature oocytes were subjected to HHP compared to the control group (61.9%). There was a tendency to obtain a lower percentage of blastocysts (17.9%) in the exp. group compared to blastocysts from the control group (33.3%), however the differences were not statistically significant. In conclusion, we did not observe a beneficial effect of high hydrostatic pressure on the developmental competence of pig oocytes and on the percentage of embryos obtained after *in vitro* fertilization.

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REFERENCES

Romek M, Kucia M, Gajda B, Krzysztofowicz E, and Smorag Z. 2019. Effect of high hydrostatic pressure on mitochondrial activity, reactive oxygen species level and developmental competence of cultured pig embryos. *Theriogenology* 140: 99–108.

Morphological and functional adaptations to viviparity in an epizoic dermapteran, *Hemimerus talpoides* (Dermaptera, Hemimeridae)

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Viviparity (live bearing) is characterized by obligate maternal retention of developing embryos within the body cavity or modified distal part of the reproductive tract (usually termed the uterus) during gestation and culminates in live birth. Viviparity was described in several insect groups, e.g. cockroaches, flies, strepsipterans, aphids and earwigs (Dermaptera). In the last group, viviparous species belong to two families: Arixeniidae and Hemimeridae. Hemimerids are epizoic and live on giant rats in sub-Saharan Africa. Their embryos develop inside terminal ovarian follicles (the embryonic follicles) and rely solely on nutrients transferred from mother tissues. Such a reproductive strategy is termed the matrotrophy.

In the present report, we describe initial stages of *Hemimerus talpoides* development that were not analyzed before, with the use of modern techniques of light and transmission electron microscopy.

Our analyses have shown that *Hemimerus* ovarian follicular cells (FCs) are diversified into three populations: (1) spherical cells surrounding the nurse cell, (2) elongated cells separating the nurse cell from the oocyte and (3) highly columnar cells surrounding lateral aspects of the oocyte and the posterior oocyte pole. The FCs of the first two subpopulations degenerate after fertilization, whereas the FCs of third population transform, rearrange and gradually form the multi-layered wall of the incubation chamber in which the embryo develops. We demonstrate additionally that amniotic cells of the early embryo remain in a direct contact with the apical surfaces of transformed FCs. In the region of contact, short outgrowths of the amniotic cells associate with irregular surface specializations of transformed FCs. The narrow gap between amniotic and transformed FCs is loaded with vesicular structures morphologically similar to the secretory vesicles found in transformed FCs cytoplasm.

In the light of our findings, we assume that extended postfertilization activity of the follicular cells represents an adaptation to viviparity and matrotrophy in Hemimeridae. We also suggest that the outgrowths of the amniotic cells play a significant role in the nourishment of the embryo.

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The influence of inflorescence removal treatment on yield of common buckwheat (*Fagopyrum esculentum* Moench)

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Common buckwheat (*Fagopyrum esculentum* Moench) of nutritive value is a serious concern of breeders due to its low fruit (seed) yield, sometimes even not exceeding 15% despite an abundant formation of flowers through a long period. The female gametophyte failure and ovule abortion but not pollen viability, germination and supply are mostly responsible for low seed set (Słomka et al., 2017).

In the present studies, we removed inflorescence in three treatments (A – 50%, B – 75%, C – all lateral shoots) in Korona cultivar of common buckwheat to reduce the competition for photosynthates between flowers and developing seeds to find whether female gametophyte degeneration could also be associated to a strong sink restriction. Treatment A revealed the most promising results increasing: (1) the frequency of properly developed embryo sacs from 77% to 97%, (2) the average number of mature seeds *per* plant by 15%, and (3) mean mass of a seed by 25%. The estimated auxin (IAA) whose content increased by 61% in flowers of plants under A treatment suggests that IAA, as a determinant factor in the regulation of endosperm initiation and development (Figueiredo and Köhler, 2020), could improve this nutritive tissue weight. Furthermore, the higher content of measured salicylic (SA) and jasmonic (JA) acids in flowers under this treatment could serve more effective pollinators attraction (Thaler et al., 2002).

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REFERENCES

Figueiredo DD, and Köhler C. 2020. Auxin: a molecular trigger of seed development. *Genes & Development* 34: 479–490.

Słomka A, Michno K, Dubert F, Dziurka M, Kopeć P, and Płażek A. 2017. Embryological background of low seed set in distylous common buckwheat (*Fagopyrum esculentum* Moench) with biased morph ratios, and biostimulant-induced improvement of it. *Crop & Pasture Science* 68: 680–690.

Thaler JS, Karban R, Ullman DE, Boege K, and Bostock RM. 2002. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia* 131: 227–235.

Ex situ conservation of endangered *Luronium natans* (Alismataceae) using *in vitro* methods

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Luronium natans (L.) Raf (Alismataceae) is an aquatic, European endemic, perennial plant with stands in north-west Poland. Despite the strict protections of the species it is observed progressive disappearance of *L. natans* habitats. Extinction is directly affected by eutrophication, humification of lakes, improper water management and rising tourism (Szmeja and Bazydło 2005). The objective of this study was to develop a protocol for seed germination and plantlets of *L. natans* proper for reintroduction. Mature seeds were taken at late summer 2018 and 2019 from 3 local populations: Lake Dobrogoszcz, Lake Krasne, and Lake Smołowe and stratified in distilled water in 4°C for 6 months, as for Alisma described (Moravcova et al. 2001). During stratification, up to 15% of seeds were germinating in distilled water only. Nongerminating seeds were then treated with 400 mg/l gibberellic acid and subsequently transferred to wet soil or after surface sterilization to *in vitro* culture. The seeds placed at the beginning into soil did not germinate. Best results for surface sterilization were obtained using 1% v/v sodium hypochlorite for 30 seconds. For breeding of plantlets best results were noted using 1/8 Hoagland medium (with 1/4 FeEDTA with 0,05% PPM, pH 5,8 (18% efficiency). Seeds then were germinating after 2–20 days. After 7–10 days of culture primary root and first leaf were observed. Seedlings increased the number of leaves and their size further, reaching about 4 cm in 60 days and were then transferred to *ex vitro* conditions at various stages of development (with 77% efficiency). Highest number of living plantlets in soil, ready for reintroduction, was obtained from Lake Krasne (35%). Optimized and effective protocols of breeding provide a valuable contribution to the ex situ conservation of L. natans.

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REFERENCES

Moravcová L, Zákravský P, Hroudová Z. 2001. *Folia Geobotanica,* 36(2): 131–146.

Szmeja J, Bazydło E. 2005. *Acta Societatis Botanicorum Poloniae.* 74(3): 253–262.

Perinatal treatment with the broad spectrum antibiotic enrofloxacin aggravates contact sensitivity in adult mice

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Antibiotics while eliminating pathogens, also partially deplete naturally existing commensal bacteria. Antibiotic-induced dysbiosis may contribute to the observed rise in the "immune-mediated" diseases including autoimmunity and allergy.

Oral treatment with broad-spectrum antibiotic enrofloxacin during gestation and breastfeeding or breastfeeding or gestation alone was used to evaluate if antibiotic exposure early in life will modulate contact sensitivity (CS) in adult mice.

Here, we demonstrated that enrofloxacin treatment during gestation and breastfeeding, but not during pregnancy or breastfeeding alone, aggravated CS reaction in adult mice measured by ear swelling. This data correlates with increased myeloperoxidase (MPO) activity in the ear extracts and elevated production of IL-6 and IL-17A by auricular lymph node cells (ELNC), and were not influenced by food consumption and body weight. In each dosing regimen, enrofloxacin treatment reduced the relative abundance of *Enterococcus* spp but did not influence the relative abundances of *Lactobacillus*, *Clostridium coccoide*s (cluster XIVa), *Clostridium coccoides – E. rectale* (cluster XIVab), *Bacteroidetes, Clostridium* (cluster I) and segmented filamentous bacteria (SFB). However, prolonged enrofloxacin-treatment during both gestation and breastfeeding specifically decreased the relative abundance of *Clostridium* cluster IV.

Our data show that long-term perinatal enrofloxacin treatment, enhance CS in adult mice. As a consequence of the long-term perinatal enrofloxacin treatment, intestinal dysbiosis is induced, characterized by decreased levels of anti-inflammatory *Clostridium* cluster IV, altering T-cell-dependent immune responses and CS susceptibility.

Ovary ultrastructure in rhyacodriline oligochaetes (Clitellata: Rhyacodrilinae) – preliminary data

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Clitellate annelids (Clitellata) comprise a group of hermaphroditic segmented worms equipped with a saddle (clitellum). Subfamily Rhyacodrilinae contains numerous genera of marine and freshwater species with a cosmopolitan distribution and together with several other subfamilies constitutes the family Naididae (sensu Erséus et al., 2002). The molecular phylogenetic analyses revealed that several rhyacodriline genera are closely related to the subfamily Naidinae (Erséus et al., 2017). As in other clitellates, the organization of microdrile reproductive systems is an important diagnostic feature with many taxonomically informative characters. On the other hand, the histological and ultrastructural data about gonad structure and gametogenesis of microdriles are fragmentary. The ovary organization and oogenesis have been never studied in Rhyacodrilinae, thus the aim of this repot was to provide such preliminary ultrastructural data.

In the studied cf. *Peristodrilus montanus* (Hrabe, 1962), we found ovaries composed of a dozen or so cysts of interconnected germ-line cells. Additionally, some somatic cells surround germ line cysts and merge them into one organ. The center of each germ-line cyst is occupied by a central cytophore to which each clustering cell is connected by one intercellular bridge. Such cyst organization is typical for all clitellates studied to date. Within ovaries cells interconnected into a given cyst develop in full synchrony and are morphologically identical. However, ovaries are polarized, i.e. there is a gradient of cysts development along the long ovary axis – at the apical ovary tip cysts with oogonia occur, whereas the cysts located at the distalmost end of the ovaries contain germ cells in diplotene. Within ovaries no specialization of germ cells into nurse cells and oocytes was observed. Ovary organization in *P. montanus* is more similar to ovaries found in Naidinae than to ovaries found in Tubificinae.

REFERENCES

Erséus C, Källersjö M, Ekman M, and Hovmöller R. 2002. 18S rDNA phylogeny of the Tubificidae (Clitellata) and its constituent taxa: Dismissal of the Naididae. *Molecular Phylogenetics and Evolution* 22: 414–422.

Erséus C, Envall I, De Wit P, and Gustavsson LM. 2017. Molecular data reveal a tropical freshwater origin of Naidinae (Annelida, Clitellata, Naididae). *Molecular Phylogenetics and Evolution* 115: 115–127.

Micro-CT study on the male reproductive system of the model organism pea aphid *Acyrthosiphon pisum* (Insecta, Hemiptera, Aphididae)

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The pea aphid complex [*Acyrthosiphon pisum* (Harris, 1776)], representing tribe Macrosiphini of the subfamily Aphidinae (Hemiptera, Aphididae), comprises 11–15 distinct host races, associated with different Leguminosae (Fabaceae). It is a globally distributed key pest of the cultivated and wild legumes, listed among the 14 aphid species of the greatest economic importance, able to transmit several serious viral diseases.

As other aphid species, the pea aphid is characterized by the complex life cycle, i.e. cyclical parthenogenesis. However, the unique feature of *A. pisum* is the presence of both winged and wingless males. As part of a multidisciplinary project on the pea aphid biology and the influence of biogenic amines on the development and functioning of the reproductive system on this aphid species, the aim of this study is to present a detailed anatomy of the male reproductive system, using a non-destructive method of micro-computed tomography (micro-CT).

The isolated colony of *A. pisum*, representing the pea biotype, were reared in plastic cages on *Pisum sativum var* Tarchalska as the host plant in the climatic chamber KKS 240/240 TOP+ with a phytotron system (POL-ECO-APARATURA SP.J., Wodzisław Śląski, Poland) in the conditions of a short day photoperiod of 8:16 D/N, temperature 15°C (±1°C) and humidity 70% (±10%) in which they produced a sexual generation, including males.

Insects, after contrasting, were scanned with an Xradia MicroXCT-400 X-ray imaging system (Carl Zeiss Microscopy GmbH), at 40 kV and 250 µA. For each sample, 900 projection images were recorded with an exposure time of 10s and a magnification objective of 20×. Tomography projections were reconstructed by using the XM-Reconstructor software (Carl Zeiss Microscopy GmbH). All scans were performed by using Binning 2 and subsequently reconstructed by using Binning 1 to avoid information loss. Voxel size was the same for all samples (1.5×1.5×1.5 µm).

The study summarizes current knowledge on the pea aphid male reproductive system and fill gaps regarding the anatomical morphology of the species studied.

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Expression of the miRNA biogenesis components and global DNA methylation in corpora lutea of adult pigs neonatally exposed to methoxychlor

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The environmental toxicants are known to induce long-term impact on the female reproduction via epigenetic mechanisms, including DNA methylation and microRNA (miRNA) expression. Methoxychlor (MXC) is an organochloride insecticide and one of the endocrine-disrupting chemicals with mixed activity: estrogenic, antiestrogenic and antiandrogenic. The usage of the MXC as a pesticide was banned in the USA and EU, however, this compound persists in the environment. It was shown that fetal and neonatal MXC exposure affected methylation of various genes in the adult rat ovary (Zama and Uzumcu, 2009). Thus, the aim of this study was to determine the effect of neonatal exposure to MXC on global DNA methylation, and the expression of DNA methyltransferases as well as proteins involved in miRNA biogenesis in corpora lutea (CLs) of adult pigs. Piglets were injected with MXC (20 μg/kg body weight) or corn oil (controls) between postnatal days 1 and 10 (n=5/each group). CLs were excised from sexually mature gilts between days 8 and 11 of the estrous cycle and examined for global DNA methylation and abundance of proteins related to DNA methylation (DNMT1, DNMT3A, DNMT3B) as well as miRNA biogenesis (DROSHA, XPO5, DICER1, AGO2) using ELISA, Western blot and immunohistochemistry. All data were analyzed using Mann-Whitney U-test. MXC significantly increased global DNA methylation level (P<0.05) and DNMT1 protein abundance (P<0.05) in luteal tissue. In addition, DNMT3A protein abundance tends to be greater (P=0.06), while XPO5 protein abundance tends to be lower (P=0.06) in the MXC-treated group. All examined proteins were localized in the both large and small luteal cells and no changes were observed in MXC-treated group as compared to the controls. Concluding, the changes in DNA methylation seem to be a part of the regulatory network that mediates long-term MXC effects on CL function in pigs.

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REFERENCES

Zama AM, and Uzumcu M. 2009. Fetal and neonatal exposure to the endocrine disruptor methoxychlor causes epigenetic alterations in adult ovarian genes. *Endocrinology* 50: 4681–4691.

Retention of mRNA encoding Sm proteins during meiotic division in Larch

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Newly synthetized mRNAs usually undergo co-transcriptional proccessing. This enables immediate transport of mRNA to the cytoplasm, where translation and synthesis of proteins occur. Recent researches show however that in some circumstances mRNAs are retained in the cell nucleus. The retention of this transcripts can be temporal or permanent. Retained transcripts were observed in the nucleoplasm, nuclear pores and nuclear bodies such as speckles and paraspeckles. So far there were no observe retention of mRNAs in the Cajal bodies (CB). Many mechanisms have been known to cause mRNAs retention, but little is they known in higher plant cells. During meiotic division in Larch mRNAs are localize in the nucleoplasm and Cajal bodies. We want to know what directed retained mRNA into the CBs. One of the mechanisms that can cause retention is intron retention (IR) therefore we performed the localization of introns in mRNA coding for SmD1, SmD2 and SmG proteins in larch meiocytes. It has been shown that the presence of one or two introns can result in retention of the entire transcript. Transcript coding for SmD1 protein has two introns and both introns are retained. This transcript was observed both in nucleoplasm and Cajal bodies. Transcript coding for SmD2 has three introns and only unspliced third intron makes retentions in Cajal bodies. The first and second introns are quickly spliced after mRNA synthesis. We performed molecular study of transcript coding for SmG protein. This mRNA has three introns and only one intron seemed to be IR and cause retention in Cajal bodies. Others intron are spliced after synthesis mRNA. There is still a lack of information about specific factors directly involved in directing pre-mRNA to Cajal's bodies, and this will be the subject of further research.

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The Balbiani body and oocytes asymmetry in largescale yellowfish *Labeobarbus marequensis* (Cypriniformes)

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The Balbiani body (Bb) is present in oocytes of a large number of species of Teleostei. However, the ultrastructure and changes in its localization in the cytoplasm (ooplasm) during growth of oocytes have been investigated thoroughly only in a limited number of species. Here, the Bb and asymmetry of the ooplasm in the largescale yellowfish *Labeobarbus marequensis* (Cypriniformes) is described. *L. marequensis* is native in south of Africa and is hexaploid. Previously, it was ascribed to the genus *Barbus*. Currently, the hexaploid *Barbus* species are placed in the genus *Labeobarbus* (Yang et al., 2015).

Ovaries of *L. marequensis* contain germinal epithelium, nests and follicles that comprise diplotene stage oocytes (primary growth, previtellogenic). Each follicle is composed of oocyte surrounded by follicular cells and a basal lamina covered by thecal cells. The oocytes nuclei contain lampbrush chromosomes, nuclear bodies and a fibrillar material in which multiple nucleoli arise. The ooplasm contains the Bb composed of nuage aggregations, rough endoplasmic reticulum (RER), mitochondria and complexes of mitochondria with cement. Initially, the Bb is present in the ooplasm close to the nucleus (stages 1, 2). Later, it expands towards the plasma membrane (oolemma) and the yolk nucleus which consists of nuage, mitochondria and numerous vesicles (RER vesicles, lysosome-like organelles and autophagosomes) arises in the Bb (stage 3). Then, the components of the Bb become dispersed in the whole ooplasm. Contrary, the yolk nucleus is located at the future vegetal region (stage 4). During the final step of primary growth, the cortical alveoli arise and are distributed evenly in the ooplasm (stage 5). The eggshell is composed of two egg envelopes that are pierced by numerous pore canals (zona radiata). The external one is covered with protuberances (stage 5). In *L. marequensis* no lipid droplets are present in the ooplasm during primary growth.

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REFERENCES

Yang L, Sado T, Vincent Hirt M, Pasco-Viel E, Arunachalam M, Li J, Wang X, Freyhof J, Saitoh K, Simons AM, Miya M, He S, and Mayden RL. 2015. Phylogeny and polyploidy: Resolving the classification of cyprininae fishes (Teleostei: Cypriniformes). *Molecular Phylogenetics and Evolution* 85: 97–116.

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6. Invited reviews are mostly of limited scope on timely subjects written for a general, well-informed audience. Invited reviews are solicited by the Editor. Ideas for unsolicited reviews should be discussed with the Editor. They are subject to the usual review procedure.
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   Examples of style for references:
   a) citations of journal papers:
   Palmer TP. 1962. Population structure, breeding system, interspecific hybridization and alloploidy. *Heredity* 17: 278-283.
   Chen BY, Heneen WK, Simonsen V. 1989. Comparative and genetic studies of isozymes in resynthesized and cultivated *Brassica napus* L., *Brassica campestris* L., and *B. alboglabra* Baitey. *Theoretical and Applied Genetics* 77: 673-679.
   b) citations of books, congress proceedings, theses:
   Bergrren DJ. 1981. *Atlas of Seeds*, part 3. Swedish Museum of Natural History, Stockholm.
   Bing D, Downey RK, Rakow GFW. 1991. Potential of gene transfer among oilseed *Brassica* and their weedy relatives. *Proceedings of the GCTRC Eighth International Rapeseed Congress*, 9-11 July 1991, 1022-1027. Saskatoon, Saskatchewan.
   Romeo JT. 1973. A chemotaxonomic study of the genus *Erythrina* (Leguminosae). Ph.D. disseration, University of Texas, Austin, TX.
   c) citations of articles and chapters from books:
   Phillips RL. 1981. Pollen and pollen tubes. In: Clark G [ed.], *Staining Procedures*, 61-366. Williams and Wilkins, Baltimore, MD.
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