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PLENARY LECTURES

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From larch to frog

Anna Noble

University of Portsmouth, School of Environmental and Life Sciences, European Xenopus Resource Centre, Portsmouth PO1 2DY, UK

The aquatic amphibian *Xenopus* has long been established as a powerful model organism in developmental and cell biology, providing unique advantages for studying vertebrate embryogenesis, gene function and disease mechanisms (Horb et al., 2022). Its external development, large embryos and ease of manipulation make it well suited to modern genetic studies and engineering approaches, reinforcing its continued relevance in both fundamental and translational research.

Key methodologies for genetic manipulation in *Xenopus* include transgenesis using the I-SceI meganuclease system (Love et al., 2011, Noble et al., 2022) and targeted mutagenesis via CRISPR/Cas technologies (Macken et al., 2021, Abu-Daya et al., 2023). These approaches enable the efficient generation of stable transgenic lines and (precise) genome editing, significantly expanding the model's utility for functional genomics and allowing gene disruption and the modelling of human disease variants.

In addition, centralised research infrastructures play an important role in supporting the global *Xenopus* community. Dedicated stock centres and integrated knowledgebases, such as Xenbase (Horb et al., 2019), provide access to curated genome data, protocols and biological materials. The European *Xenopus* Resource Centre (EXRC, UK) contributes essential resources (e.g. live animals, oocytes, embryos, fresh testes, frozen sperm, molecular reagents), as well as training, technical expertise and a "research hotel" environment that facilitates visiting researchers conducting experiments on site. Furthermore, XenMD, a spin-off initiative focused on translating *Xenopus*-based discoveries into biomedical applications, highlights the model's growing impact beyond basic research.

Together, these advances underscore the enduring impact of *Xenopus* in genome engineering and its growing contribution to translational science.

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What is the future of plant embryology? Contemporary dilemmas and research problems

Monika Kwiatkowska

Department of Plant Cytology and Embryology, Institute of Botany, Jagellonian University, Gronostajowa 9, 30-387 Cracow, Poland, e-mail: monika.m.kwiatkowska@uj.edu.pl

One of the fundamental characteristics of living beings is the ability to reproduce. This process not only ensures the continuity of life *sensu lato* but – through the production of new individuals – guarantees the survival of a given species.

Flowering plant embryology is the science that studies the reproductive processes, including sexual and apomictic reproduction in Angiosperms. Our focus is the transition from the sporophyte to the gametophyte phase. The gametophyte – a phase entirely dependent on the sporophyte in the course of evolution. The process of flowering and fruiting includes key stages such as sporogenesis, gametogenesis, double fertilization, seed formation, and fruit development. Meiosis during sporogenesis plays a key role in biodiversity and evolution. Therefore, this field of science is important in many aspects. Observing the development of embryology, we can distinguish stages, which is directly related to the development of research techniques. Thus, descriptive, comparative, experimental, and molecular embryology can be distinguished. The initial stage of this discipline's development was descriptive embryology, which described the development of generative structures and the structure of the flower, and described the course of these processes species by species. Its heyday occurred from the second half of the 19th century to the first half of the 20th, when this science functioned as an independent field. The results obtained during this period constitute the foundation of the discipline, remaining essential and relevant knowledge. On this basis, comparative embryology systematized these processes according to the taxonomic position of plants. The next stage, associated with the development of electron, fluorescence, and confocal microscopy techniques, as well as immunohistochemical methods, allowed for the exploration of the ultrastructure of generative organs. Research in this area is currently underway. Simultaneously, experimental embryology developed, enabling the isolation of structures and developmental manipulations, such as the production of haploids. This is an important branch of embryology. The next stage in the development of embryology is genetic and transcriptomic research. By sequencing the genes of model species (e.g., *Arabidopsis thaliana*), we can observe mutants and search for genes responsible for specific processes. Another breakthrough in understanding embryological processes is the analysis of single-cell transcriptomes – that is, the study of gene expression in specific cells using scRNA-seq. Due to these advanced molecular techniques, embryology, from answering the question of what process occurs and whether it occurs, also attempts to answer the question of how it occurs.

Today's embryology is an interdisciplinary science. It no longer only connects with cytology, anatomy or histology, but also with molecular genetics, ecotoxicology, biotechnology, and bioinformatics. It utilizes both classical techniques and the latest research techniques and requires collaboration with scientists from many fields.

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ORAL PRESENTATIONS

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Morphology of ovarioles, oogenesis and formation of a putative germ plasm in tubuliferan and terebrantian thrips (Thysanoptera)

Szczepan Bilinski[1], Magda Sochaczewska[1,2], Waclaw Tworzydlo[1]

[1]*Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Krakow, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland, e-mail: szczepan.bilinski@uj.edu.pl*

Morphology of ovaries and ovarioles as well as the ultrastructure of subsequent stages of oogenesis have been analyzed in three species representing both thysanopteran subtaxa: Terebrantia (*Thrips* sp. and *Frankliniella occidentalis*) and Tubulifera (*Haplothrips leucanthemi*). Histological methods, scanning and transmission electron microscopy have been used in these studies. Our analyses have revealed that the morphology of ovaries in studied terebrantians *vs* tubuliferans is disparate. The main difference concerns the morphology of the anterior ovary/ovariole region. Whereas in the tubuliferan, *H. leucanthemi*, terminal filaments and germaria of all four ovarioles constituting a given ovary are fused and form single common structures (a common terminal filament and a germarial chamber, respectively), in both studied terebrantians they are individual - as in other insect taxa. On the grounds of our studies and a survey of literature, we suggest that the fusion of the anterior ovariole parts represent an exceptional trait, characteristic of tubuliferan thrips only. On the contrary, the course of oogenesis in analysed species is remarkably similar and reveals at least one prominent feature: presence of a distinct perinuclear nuage layer tightly surrounding oocyte nuclei (germinal vesicles). As oogenesis progresses, this layer undergoes noticeable and species-specific transformation. In both thripid species the layer becomes progressively thinner and eventually disappears, suggesting that its constituents dissolve in the ground cytoplasm. In contrast, in *H. leucanthemi* the blocks building the perinuclear nuage layer detach from the germinal vesicle and split into smaller fragments that are transferred towards the oocyte periphery. We show additionally that after reinitiation of the first meiotic division in representatives of both thysanopteran subgroups, a small yolk free island of cytoplasm arises at the posterior oocyte extremity. We interpret this island as the putative polar/germ plasm.

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UNRAVELING THE MYSTERY OF RETINAL DEVELOPMENT IN THE BROWN ANOLE (*ANOLIS SAGREI*) – HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES

JULIA BOROWSKA[1], PAWEŁ KACZMAREK[1], JOLANTA RAJCA[2], WERONIKA RUPIK[1]

[1]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland, e-mail: julia.borowska@us.edu.pl; weronika.rupik@us.edu.pl*

[2]*Spin-Lab Centre for Microscopic Research on Matter, University of Silesia in Katowice, Katowice, 75 Pułku Piechoty 1A, 41-500 Chorzów, Poland, e-mail: jolanta.rajca@us.edu.pl*

Retinal differentiation processes in reptilian species are poorly described compared with those of other vertebrates. The brown anole (*Anolis sagrei*), a representative of squamates, is a diurnal, bifoveated lizard with high-acuity vision, which enables it to actively hunt and communicate effectively. Lizards are promising models for structural and comparative investigations. The uniqueness of the squamate retina is reflected in the presence of a 'simplex retina', with only one type of light-sensitive cells (cones or rods). In fact, there are some mixed ultrastructural and molecular features suggesting evolutionary transitions between diurnal and nocturnal activity. In contrast, other vertebrates possess a 'duplex retina' containing both types of cells (cones and rods). Furthermore, in squamates, photoreceptors exhibit a wide range of forms and show a great variety in their inner segments. The aim of this study was to investigate the sequence of retinal layer differentiation and to identify the main types of retinal cells, their order of appearance, and characteristic features. Retinogenesis in the brown anole was studied using light microscopy, transmission electron microscopy (TEM), and X-ray microtomography (micro-CT). Embryo isolation was performed at regular intervals prior to hatching. The age of embryos was calculated using specific developmental tables (Sanger et al., 2008). The results revealed that at the beginning, the retina consists of a pseudostratified neuroepithelium. It remains undifferentiated, with no distinct layers. In the middle of embryonic development, the layers begin to form. Then, in the second half of development, photosensitive cells appear, and distinct layers differentiate. In late embryos, the retina is fully developed and is composed of seven layers. This study allows understanding the relationship between retinal structure and the visual requirements of species with different circadian activity patterns for inhabiting certain ecological niches. Moreover, it sheds light on the evolution of vertebrate visual systems and fills gaps in comparative neurobiology and anatomy.

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OOGONIA DO NOT CONTRIBUTE TO FUNCTIONAL OOCYTE PRODUCTION IN ADULT FROGS

MAGDALENA CHMIELEWSKA, JAGIENKA APOLINARSKA, MARIA OGIELSKA, BEATA ROZENBLUT-KOŚCISTY

Amphibian Biology Group, Department of Evolutionary Biology and Conservation of Vertebrates, Faculty of Biological Sciences, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: magdalena.chmielewska@uwr.edu.pl

In juvenile ovaries of anuran amphibians mitotic activity of primary oogonia establishes a pool of diplotene oocytes sufficient for all breeding seasons during female's lifespan (Ogielska et al., 2013). In mature ovary, oogonia are still present within residual clusters of early germ cells, known as germ patches, located within the ovarian cortex. We aimed to determine whether oogonia present in the germ patches retain mitotic activity and whether early meiotic cells contribute to the diplotene oocyte pool in adult females of the grass frog *Rana temporaria*. In paraffin section of the ovaries, we identified all classes of germ cells using the germ cell marker VASA. Juvenile females exhibited more numerous germ cell patches and a higher number of oogonia than adults. Mitotically active oogonia (approximately 0.5% of all cells) and leptotene-stage oocytes were observed only in juveniles, together with small diplotene oocytes, which were rare in adults. Replicative activity, assessed by BrdU incorporation, was observed in somatic cells and oogonia in larval and juvenile ovaries; however, no BrdU labeling was detected in oogonia and smallest diplotene oocytes from the germ patches in adult ovaries. Proliferating cells nuclear antigen (PCNA), indicating ongoing DNA replication, was detected in some oogonia and was more frequent in juveniles than in adults. Numerous degenerating cells were observed in the germ patches of adult ovaries. We found that oogonia, meiotic cell nests, and diplotene oocytes underwent apoptosis, as indicated by a high level of active caspase-3 expression.

Conclusion: although some oogonia in germ patches of adult females retain limited replicative activity, they rarely enter mitosis or produce meiotic cells. This is further supported by the low numbers of small diplotene oocytes in adult ovaries, many of which undergo apoptosis and therefore do not contribute to the production of new oocytes in adult females.

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An insight into ovary of Hydrachnidia: 3D reconstruction of the germline cyst

Anna Derdak[1], Joanna Mąkol[2], Karol Małota[3,4], Izabela Jędrzejowska[1]

[1]*Department of Animal Developmental Biology, University of Wroclaw, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: anna.derdak@uwr.edu.pl, izabela.jedrzejowska@uwr.edu.pl*

[2]*Department of Animal Biology and Ecology, Wroclaw University of Environmental and Life Sciences, Kożuchowska 5B, 51-631 Wrocław, Poland, e-mail: joanna.makol@upwr.edu.pl*

[3]*Institute of Biology, Biotechnology and Environmental Protection, University of Silesia, Jagiellońska 28, 40-032 Katowice, Poland, e-mail: karol.malota@us.edu.pl*

[4]*SPIN-Lab Centre for Microscopic Research on Matter University of Silesia, 75 Pułku Piechoty 1a, 41-500 Chorzów, Poland*

The structure of the female gonads in mites exhibits remarkable diversity. They can be categorized as either panoistic, where oocytes develop independently of nurse cells, or meroistic, where oocytes receive support from nurse cells during their growth. This variation stands in contrast to other chelicerates, which typically have a more uniform panoistic ovary. Both types of ovaries are found in the two mains lineages of mites, Acariformes and Parasitiformes. The organization of meroistic ovaries in these lineages suggests that this trait evolved independently several times.

Hydrachnidia (Chelicerata, Acariformes), commonly known as water mites, represent one of the most diverse and widespread groups of freshwater arthropods worldwide. Despite their abundance, data on their ovary structure is scarce and primarily based on studies from the early 20 c. This study aimed to analyze the ovaries of adult females of various taxa aggregated in water mites.

Representatives of five families (Arrenuridae, Hydrachnidae, Hydrodromidae, Pionidae, Limnesiidae) of water mites were examined using light-, confocal/fluorescence-, and transmission electron microscopy. The analyses were further enhanced by serial block-face scanning electron microscopy (SBEM), which facilitated the three-dimensional reconstruction of the germline cysts.

Our study is the most comprehensive to date regarding the female gonads in Hydrachnidia and is the first to report the meroistic ovaries within this group. The ovary contains numerous germline cysts that differentiate into oocytes, which bulge at the surface of the ovary, and nurse cells located within the ovarian wall. The nurse cells are significantly larger and have highly branched, polyploid nuclei rich in condensed chromatin. Each cell within a cyst is connected to a central cytoplasm via elongated trophic cords, which transfer macromolecules and organelles from the nurse cells to the oocytes in a microtubule-dependent manner.

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How diversity of reproductive strategies may be reflected in the testis structure and in the spermatogenesis process in some bony tongue fish?

Anna M. Dymek[1], Anna Pecio[2], Frank Kirschbaum[3,4], Ralph Tiedemann[4]

[1]*Department of Small Livestock Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland, e-mail: anna.dymek@iz.edu.pl*

[2]*Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, Kraków 30-387, Poland, e-mail: anna.pecio@uj.edu.pl*

[3]*Unit of Evolutionary Biology/Systematic Zoology, University of Potsdam, Karl-Liebknecht-Strasse 24-25, Haus 26, Potsdam 14476, Germany*

[4]*Unit of Biology and Ecology of Fishes, Faculty of Life Sciences, Humboldt University of Berlin, Philippstr. 13, Haus 16, Berlin 10115, Germany*

Teleosts exhibit significant variation in their reproductive biology, including, but not limited to, testis structure, types of spermiogenesis, types of sperm, and modes of fertilization. One of the earliest Teleostei lineages, Osteoglossomorpha, especially order Osteoglossiformes, with nearly 300 species, reflects many of these various traits (Hilton and Lavoue, 2018). This group encompasses species that demonstrate various modes of fertilization, including internal or external fertilization. The species possess either single or paired gonads and produce introsperm or uniflagellate, biflagellate, or aflagellate aquasperm.

A comparative analysis of the testes of *Pantodon buchholzi*, *Notopterus notopterus*, and *Campylomormyrus compressirostris* was conducted using light and electron microscopes (scanning and transmission). This analysis enabled the identification of both similarities and differences in testis structure and spermatogenesis of these species.

The results demonstrated that paired gonads are exclusively characterized by *P. buchholzi*, while all three studied species exhibited a clear compartmentalization into two distinct regions: the anterior spermatogenic part and the posterior aspermatogenic part. Notably, in *P. buchholzi*, the aspermatogenic part also functions as an additional gland. The tubular testes of all analyzed species exhibit anastomosing tubules with cysts containing germinal cells at varying stages of development. *P. buchholzi* forms introsperm, which subsequently form spermatozeugmata, which are stored in additional gland until insemination occurs. It has been observed that *N. notopterus* and *C. compressirostris* form aquasperm. However, in the latter case, the sperm are found to be devoid of a flagellum.

A comparative analysis of the three species reveals both common elements of testicular organization and distinct differences resulting from distinct reproductive strategies.

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ADAPTATION FOR MATROTROPHY IN *CHEIRIDIUM MUSEORUM*, ONE OF THE SMALLEST PSEUDOSCORPIONS (CHELICERATA, PSEUDOSCORPIONES, CHEIRIDIIDAE)

ARNOLD GARBIEC, IZABELA JĘDRZEJOWSKA

Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wrocław, Sienkiewicza 21, Wrocław, Poland, e-mail: arnold.garbiec@uwr.edu.pl

Cheiridiids are one of the smallest pseudoscorpions, which display significant features of miniaturization in the structure of female reproductive system. These include a spatially restricted germarium located in the central part of the exceptionally short ovary and a limited number of oocytes (up to six) that develop during each ovarian cycle. The oocytes are attached to the ovary surface by means of stalks, which are thin and elongated (Jędrzejowska et al., 2021). Cheiridiids, like other pseudoscorpions are matrotrophic, and feed their embryos on the nutritive fluid produced in the female reproductive system.

The aim of our study was to investigate features involved in adaptations for matrotrophy in the female reproductive system in *Cheiridium museorum*. We also wanted to check whether those features could be affected by characters related to adaptations for miniaturization.

We showed that in *C. museorum*, the nutritive fluid for developing embryos is produced in the ovaries and the oviducts by the polyploid and hypertrophic epithelial cells. We also showed that the stalk cells do not produce the nutritive fluid, which is the case of the more advanced matrotrophic species of the families Cheliferidae and Chernetidae (Cheliferoidea) (Jędrzejowska et al., 2020, Garbiec et al., 2022). Furthermore, oocytes accumulate only a negligible amount of yolk during vitellogenesis.

The results indicate that the mode and efficiency of the nutritive fluid synthesis are similar to that described in Neobisiidae. However, the negligible amount of yolk in the oocytes resembles cheliferids and chernetids. It indicates that *C. museorum* displays intermediate characteristics between neobisiids and cheliferoids in adaptations for matrotrophy. These adaptations do not seem to be affected by the small body size.

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Multigenerational impact of elevated crossover rates on genome stability and short-term evolutionary dynamics in *Arabidopsis thaliana*

Monika Gazecka, Franz Boideau, Piotr A. Ziółkowski

Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, ul. Wieniawskiego 1 61-712 Poznań, Poland, e-mail: monika.gazecka@amu.edu.pl, franz.boideau@gmail.com, piotr.ziolkowski@amu.edu.pl

Meiotic crossovers are maintained at very low levels, typically 1 to 3 per chromosome pair per meiosis, despite the lack of an obvious impact on the course of meiosis. Our recent unpublished work shows that simultaneously inactivating the major crossover pathway (the ZMM pathway), and the anti-recombinational helicase RECQ4 dramatically increases the number of crossovers (by 5-fold), while maintaining relatively high plant fertility. This modest decrease in fertility relative to the increase in crossover frequency may indicate that the effects of elevated crossover rates are not immediate, but instead emerge after multiple sexually propagated generations. It is possible that chromosomal rearrangements accumulate over time, gradually leading to loss of genome stability and, ultimately, dysfunction.

The aim of my PhD project is to investigate how increased crossover frequency affects plants in terms of genome stability, accumulation of point mutations, and fertility.

Individual seeds of two *Arabidopsis thaliana* accessions (Col and Ler) carrying different hyper-recombinant mutations will be propagated for 20 generations of self-pollination. Parental plants and plants from generations #5, #10, #15, and #20 will be used to measure fertility, allowing me to assess short- and medium-term effects on reproductive capacity. Generations #0, #5, and #20 will be sequenced using ONT to examine the accumulation of structural genomic changes over time.

The results of this project will provide insight into the roles of the ZMM pathway, the MutLγ complex, the synaptonemal complex, and DNA helicases in maintaining genome stability. They will also allow me to determine the impact of a substantial increase in recombination frequency on fertility and genome stability in a multigenerational context.

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AN APPLICATION OF MRI IMAGING FOR ANALYSIS OF TOPOGRAPHY CHANGES OF ORGANS IN THE ABDOMINAL CAVITY OF THE SHORTHAIR CAT DURING PRENATAL DEVELOPMENT

HANNA JACKOWIAK[1], TOMASZ ZALEWSKI[2], KINGA SKIERESZ-SZEWCZYK[1], EWELINA BASIŃSKA[1], BARBARA KRUSZYŃSKA[1]

[1]*Department of Anatomy, Histology and Embryology of Animals, Poznan University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland, e-mail: hanna.jackowiak@up.poznan.pl*

[2]*NanoBioMedical Centre, Adam Mickiewicz University, Wszechnicy Piastowskiej 3, 61-614 Poznań, Poland*

During prenatal development, the spatial arrangement of organs in the abdominal cavity of mammals undergoes significant changes. This study aims to evaluate topographical changes in the developing abdominal organs of the domestic cat. The research material consisted of formalin-fixed feline fetuses, 28th- 62nd days post conceptionem (p.c.). Magnetic resonance 2D and 3D images were collected using an MRI scanner (Agilent 9,4 T), and a 3D reconstruction of the abdominal cavity scans was performed in AMIRA software. According to Polish law and EU directives, the research did not require the approval of the Local Ethical Committee.

Digestive system. Between the 29th and 31st day p.c., the most voluminous organ is the liver, fulfilling hematopoietic function. The second rotation of the stomach is observed. Between the 29th and 31st day p.c., the lobulation of the liver occurs t. Until the 31st day p.c., the short intestinal loops are positioned within the liver lobes. The growth of intestinal loops and the formation of an umbilical hernia occur around the 38th day p.c.. An arrangement of intestines only in the abdominal cavity is established by the 42nd to 50th day p.c.

Urinary system. The mesonephroi occur in the dorsal part of the abdominal cavity, and finally regress up to 50 day p.c. The kidneys are positioned medially above the mesonephroi and from day 38 p.c., laterally in the abdominal cavity. From 42nd day p.c. the right kidney lies dorsally to the right lobe of the liver, while the left kidney is located below the body of the stomach.

Our MRI analyse present dynamic spatial topography and organogenesis of the abdominal organs in the cat as a basis for detailed microscopic studies.

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Mitochondria in oocytes and early embryos in chernetid pseudoscorpions – preliminary studies

Izabela Jędrzejowska[1], Jana Christophoryová[2], Arnold Garbiec[1]

[1]*Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: izabela.jedrzejowska@uwr.edu.pl*

[2]*Department of Zoology, Comenius University, Ilkovičova 6, Bratislava, Slovakia e-mail: christophoryova@gmail.com*

Mitochondria are highly dynamic and multifunctional organelles responsible for ATP production, calcium homeostasis, fatty acid oxidation, regulation of cell death, and signalling pathways. Mitochondria are critical for the functioning of all cell types, and play an essential role in oocytes and embryos. The quality of mitochondria affects the oocyte growth, fertilization, and embryo development and survival.

Pseudoscorpions are small arachnids with a peculiar reproductive biology. Unlike most chelicerates, pseudoscorpions are matrotrophic animals whose embryos feed on nutrients provided by a maternal organism.

The goal of the study was to investigate the morphology and mitochondrial behavior of oocytes and early embryos in pseudoscorpions from the family Chernetidae. This family belongs to the Cheliferoidea superfamily, which is considered the most advanced of the pseudoscorpion superfamilies. Representatives of this superfamily show the highest level of specialization for matrotrophy.

Our preliminary studies carried out in the light–, electron transmission–, and confocal microscopes have shown that in chernetids the mitochondria show similarities in morphology and distribution that change during subsequent stages of oocyte growth. In early previtellogenic stages, the mitochondria accumulate in the Balbiani body, and constitute its main component. In advanced previtellogenesis, after the Balbiani body disintegration, the mitochondria populate the entire cytoplasm. During this stage, the mitochondria aggregate with a non-membrane nuage material that appears on the surface of an individual mitochondrion. In most studied chernetids, the mitochondria aggregated with nuage form complexes in which the mitochondria are arranged in stacks. These complexes remain in the ooplasm until late vitellogenic stages, and are also observed in micromeres during the early cleavage period.

Our preliminary studies indicate that aggregation of nuage with mitochondria during post Balbiani stages is a family-specific feature. We postulate that it exemplifies a strategy for storing RNA and proteins. In the case of chernetids, the material associated with mitochondria is produced by the oocytes and transferred to the embryos.

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EMBRYOLOGY OF THE PITUITARY GLAND OF THE BROWN ANOLE *ANOLIS SAGREI* (SQUAMATA: IGUANIA)

NATALIA JURAS[1], PAWEŁ KACZMAREK[1], JOLANTA RAJCA[2], WERONIKA RUPIK[1]

[1]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland, e-mail: njuras@o365.us.edu.pl, pawel.kaczmarek@us.edu.pl, weronika.rupik@us.edu.pl*

[2]*Spin-Lab Centre for Microscopic Research on Matter, University of Silesia in Katowice, Katowice, 75 Pułku Piechoty 1A, 41-500 Chorzów, Poland, e-mail: jolanta.rajca@us.edu.pl*

The pituitary gland is the main endocrine organ, a highly specialized structure present in all vertebrates. It plays an essential role in hormone secretion, reproductive cycle regulation, and neurosecretion. The overall morphology of the pituitary gland exhibits remarkable similarity across vertebrates. However, the structure itself varies greatly between species. In reptiles, at least nine morphological types of this organ have been identified, corresponding to their taxonomic classification. Squamate reptiles (lizards and snakes) are among the most diverse vertebrate clades, comprising over 12,000 extant species and providing insights into vertebrate evolution. In this study, we used embryos of the brown anole (*Anolis sagrei*), an emerging model organism widely used in developmental and evolutionary research. Nevertheless, current knowledge of the pituitary gland and brain development in this species, as well as in other squamates, is limited. Using histological serial sections and X-ray microtomography, we performed 3D reconstructions of the brain at selected developmental stages and analyzed the topology and developmental dynamics of individual brain regions, with particular emphasis on the pituitary gland. We traced the separation of the adenohypophysis from the developing oral cavity and its subsequent specialization into three main parts: the pars distalis, the pars intermedia, and the pars tuberalis, which develops from lateral outgrowths of the posterior part of the pars distalis. The developing adenohypophysis was analyzed in relation to the neurohypophysis and the rest of the diencephalon. Our results reveal complex embryonic changes leading to pituitary gland formation, characterized by a well-developed pars intermedia. By combining histological sections with detailed 3D reconstructions, we provide new insights into the spatial organization, morphology, and development of the pituitary gland in squamate reptiles.

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Evolution of vomeronasal chemoreception in squamates: insights from embryology

Paweł Kaczmarek[1], Ingmar Werneburg[2,3], Brian Metscher[4], Weronika Rupik[1]

[1]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland, e-mail: pawel.kaczmarek@us.edu.pl, weronika.rupik@us.edu.pl*

[2]*Senckenberg Center for Human Evolution and Paleoenvironment an der Eberhard Karls Universität, Sigwartstraße 10, Tübingen, Germany, e-mail: ingmar.werneburg@senckenberg.de*

[3]*Fachbereich Geowissenschaften, Eberhard Karls Universität, Hölderlinstraße 12, Tübingen, Germany*

[4]*Department of Evolutionary Biology, University of Vienna, Djerassiplatz 1 A-1030 Vienna, Austria, e-mail: brian.metscher@univie.ac.at*

Squamates are characterized by the separation of the VNO from the nasal cavity and its direct connection to the oral cavity, enabling tongue-mediated vomerolfaction. Rostrally, the lacrimal duct consistently reaches or closely approaches the VNO duct, delivering Harderian gland secretion that serves as an additional solvent for odor molecules. In addition, in lizards, the lacrimal duct is closely associated with the choanal groove, which represents a ventral remnant of the embryonic nasal cavity. This association is particularly evident in lacertids, in which both structures form the lacrimo-choanal gutter and are intimately associated, rendering them difficult to distinguish. In contrast, in adult snakes, the choanal groove is absent. Here, we present the results of our decade-long embryological investigations into the development of the naso-palatal complex across squamates, together with the only extant representative of the sister group to Squamata, the tuatara (*Sphenodon punctatus*). Using histological serial sections and 3D reconstructions based on X-ray microtomography, we found that the general pattern of naso-palatal complex development is similar across all studied squamates, except for some noticeable differences in the timing of developmental characters. Delays in the development of the lacrimal duct and choanal groove, combined with differences in their initial contact extent, may shape the lacrimo-choanal gutter in Lacertoidea. Therefore, the gekkotan-like choanal groove, confluent with the VNO duct, is likely recapitulated in all squamates, since such a condition was found in embryos of lacertids and snakes. The choanal groove was not observed to form in the tuatara, supporting the interpretation that this structure represents a synapomorphy of squamates. We suggest that the choanal groove, often overlooked in squamate chemoreception, has a dual role: guiding the lacrimal duct to the medial aspect of the VNO duct during development and contributing to the lacrimo-choanal gutter in adults, potentially facilitating chemical delivery to the VNO. We also explore its links to superficial palate morphology, tropotaxis, and vomeronasal sensory sensitivity.

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How intine wall architecture and a specialised apoplastic wall domain between the generative and vegetative cells may support rapid pollen germination in early-spring geophytes?

Małgorzata Kapusta[1], Magdalena Narajczyk[1], Bartosz J. Płachno[2]

[1]*Bioimaging Laboratory, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland*

[2]*Institute of Botany, Faculty of Biology, Jagiellonian University, 9 Gronostajowa St., 30-387 Kraków, Poland, e-mail: malgorzata.kapusta@ug.edu.pl*

Early-spring geophytes must achieve rapid pollen hydration and efficient germination within short and thermally unstable reproductive windows. In this context, the structural organisation of the pollen intine and the composition of the specialised apoplastic wall domain located between the generative and vegetative cells may be functionally important. In our studies on monocotyledonous pollen, we combined confocal immunofluorescence, immunogold transmission electron microscopy and selective cell wall probing to characterise these two domains in pollen grains of early-spring species.

In *Gagea lutea*, the intine emerged as the principal site of wall-related signals. Xyloglucan-associated epitopes recognised by LM15, LM24, LM25 and CCRC-M48 were localised within this domain, whereas LM10, LM11, LM21, LM22 and CCRC-M138 remained below the detection threshold under the applied conditions. Sequential treatment with pectate lyase and endo-β-mannanase increased the continuity and detectability of several intine-associated signals, consistent with epitope unmasking within a structurally constrained hemicellulosic matrix. This interpretation was further supported by Carbotrace staining, which highlighted a glucan-rich intine domain, particularly in the aperture region. In parallel, analyses of *G. lutea*, *Ornithogalum nutans* and *Galanthus nivalis* showed that AGP epitopes detected with JIM8 and JIM13 consistently marked the region between the generative and vegetative cells, revealing an evolutionarily conserved AGP-rich apoplastic domain, whereas the intine epitope pattern in *G. lutea* appeared less canonical for monocots and pointed to a more distinctive wall organisation in this early-spring non-grass species.

Together, these observations support the view that pollen of early-spring geophytes combines two specialised structural systems: an intine architecture enriched in digestion-sensitive wall epitopes and an apoplastic wall domain associated with the male germ unit. Although the functional consequences remain to be tested directly, both domains may contribute to the spatially coordinated onset of pollen germination under early-spring conditions.

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STRUCTURAL TRANSFORMATIONS OF THE SOMITE DURING EMBRYOGENESIS OF THE MOURNING GECKO (*LEPIDODACTYLUS LUGUBRIS*)

OLIWIA KOBĘDZA, IZABELA POTOCKA, PAWEŁ KACZMAREK, WERONIKA RUPIK

Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland,
e-mail: oliwia.kobedza@us.edu.pl, weronika.rupik@us.edu.pl

Somitogenesis is a fundamental process during early vertebrate embryonic development, as it establishes the segmental organization of the body plan. Cells derived from somites give rise to the dermis, skeletal muscles, ribs, and vertebral column. Although the mechanisms of somitogenesis are well understood in classical model organisms, information regarding this process in reptiles remains limited. Thus, this study focuses on characterizing somite differentiation at the structural and ultrastructural levels in the mourning gecko (*Lepidodactylus lugubris*), representing the Gekkota clade. This proces was performer using complementary microscopic techniques, including light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Differentiation of the presomitic mesoderm (PSM) proceeds along the cranio-caudal axis, beginning with the formation of mesenchymal somitomeres that lack visible mitotic figures. As epithelialization advances, these somitomeres transform into epithelial somites, with the most mature somites located in the cranial region. Initially, somites appear as spherical epithelial structures containing mitotic figures. Subsequently, a central cavity, the somitocoel, forms within each of them. The somitocoel divides the somite into two distinct domains: a dorsal and a ventral one. During further differentiation, the dorsal domain develops into the dermatomyotome, forming characteristic dorsal and ventral lips, while the ventral domain gives rise to the mesenchymal sclerotome. Cells originating from the dermatomyotomal lips migrate medially to form the myotome as a compact cellular layer. At this stage, the somitocoel remains present but gradually disappears as myotome formation progresses. In later stages outer cells of the dermatomyotome migrate toward the epidermis to form the presumptive dermis. Within the myotome, numerous myofibrils become evident, marking the onset of skeletal muscle differentiation. At the same time, sclerotomal cells migrate deeper into the embryonic body, contributing to the formation of vertebral and rib primordia. The results of this study showed that reptilian somitogenesis follows a highly conserved sequence of morphological events, while also providing new structural data that enrich the broader comparative understanding of vertebrate embryonic development.

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FUNCTIONAL ROLE OF VISFATIN IN EARLY EMBRYONIC DEVELOPMENT IN NORMAL-WEIGHT AND OBESE MICE REVEALED BY siRNA-MEDIATED KNOCKDOWN

PATRYCJA KUROWSKA[1], SHINNOSUKE HONDA[2], GENTA TANAKA[2], GAO YUWEI[2], NAOJIRO MINAMI[2], AGNIESZKA RAK[1], SHUNTARO IKEDA[2]

[1]*Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University in Krakow, Gronostajowa 9 street, 30-387, Krakow, Poland, e-mail: patrycja.kurowska@uj.edu.pl; agnieszka.rak@uj.edu.pl*

[1]*Laboratory of Reproductive Biology, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Sakyo Ward, 606-8502, Kyoto, Japan, e-mail: honda.shinnosuke.4x@kyoto-u.ac.jp; tanaka.genta.37w@st.kyoto-u.ac.jp; gao.yuwei.22p@st.kyto-u.ac.jp; minami.naojiro.v18@kyoto-u.jp; ikeda.syuntaro.6u@kyoto-u.ac.jp*

Reproductive success across animals is tightly linked to the metabolic status of the organism, as nutrient availability and metabolic signaling influence gamete quality and early embryonic development. Among metabolic regulators, adipokines, hormones produced by adipose tissue have emerged as important endocrine mediators connecting energy balance with reproductive processes. Visfatin/*Nampt* is an adipokine elevated in obesity that has been linked to oocyte maturation, yet its role during early embryonic development remains unclear. This study investigated the function of visfatin/*Nampt* during preimplantation development in embryos from normal-weight and high-fat diet–induced obese mice.

Embryos were collected from females maintained on either a control diet or a high-fat diet to model maternal obesity and were cultured *in vitro* under standard preimplantation culture conditions. *Nampt* expression was evaluated from the 1-cell stage to the blastocyst stage, and functional relevance was tested using siRNA-mediated *Nampt* silencing at the 1-cell stage. Embryo developmental competence was assessed by cleavage and blastocyst formation rates. Molecular effects were analyzed by qPCR of genes related to maternal effect, proliferation/apoptosis and lineage specification, immunofluorescence detection of pluripotency and differentiation markers, and transcriptomic profiling (RNA-seq) of differentially expressed genes between groups. Reproductive outcomes, including implantation rate, litter size and ovarian gene expression in offspring, were also examined.

Nampt expression decreased during early embryogenesis. *Nampt* silencing impaired blastocyst formation in embryos from obese mice and altered lineage marker expression, increasing NANOG in normal-weight embryos and reducing GATA6-positive cells in obese embryos. RNA-seq analysis revealed 73 upregulated and 24 downregulated genes associated with apoptosis, metabolism and developmental pathways. Although implantation rates and offspring numbers were not affected, progeny derived from *Nampt*-silenced embryos showed altered ovarian expression of genes involved in steroidogenesis and oogenesis.

These findings indicate that visfatin/*Nampt* contributes to early lineage allocation in a manner dependent on maternal metabolic status and may participate in embryonic programming.

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Quo vadis – plant embryology in the era of AI and new global challenges

Elżbieta Kuta[1], Aneta Słomka[1], Justyna Żabicka[1], Klaudia Sychta[1], Monika Kwiatkowska[1], Błażej Slazak[2,3], Grzegorz Migdałek[4], Piotr Żabicki[5]

[1]*Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, 9 Gronostajowa St., 30-387 Cracow, Poland, e-mail: elzbieta.kuta@uj.edu.pl; aneta.slomka@uj.edu.pl; j.zabicka@uj.edu.p;l klaudia.sychta@u.jedu.pl; monika.m.kwiatkowska@uj.edu.pl*

[2] *W. Szafer Institute of Botany, Polish Academy of Science, 46 Lubicz St., 31-512,Cracow, Poland, e-mail: b.slazak@botany.pl*

[3] *Department of Pharmaceutical Biosciences, Pharmacognosy, Uppsala University, Biomedical Centre (BMC) 574, 75123, Uppsala, Sweden*

[4]*Institute of Biology and Earth Sciences, University of the National Education Commission, Krakow, 2 Podchorążych St., 30-084 Cracow, Poland, e-mail: grzegorz.migdalek@uken.krakow.pl*

[5]*Cracow, Poland, e-mail: piotrek.zabicki@alumni.uj.edu.pl*

Plant embryology is a field I have been involved in for almost 60 years (E. Kuta master's thesis, the embryology of *Sonchus oleeraceus*, 1969). I started with classical embryology (light microscopy), using in following years electron (SEM, TEM), confocal microscopy and immunocytochemical techniques in cooperation with young coworkers. We did research also in experimental embryology (*in vitro* induction of autonomous endosperm, somatic embryogenesis) in embryotoxicology (the effect of heavy metals on plant reproduction), the role and distribution of cyclotides in the cells of the embryo and endosperm. This allows me to start a discussion and ask a fundamental question about the direction of plant embryology research in the coming years and beyond (2026-2035).

We used AI to ask questions related to the research we have conducted and which are continue in our Department: GPT 5.2 extended question: "*Quo vadis* - Plant embryology in the era of AI and new global challenges: habitat destruction (peatlands, wetlands), pollution from plastics, herbicides, heavy metals, the subsequent extinction of plant species" GPT 5.2 extended answer: *Quo vadis?* Plant embryology must become an "early warning" science, diagnosing and restoring plant reproductive capacity. Embryology as a "sensor" of environmental quality, not only describing development (embryos, endosperm, pollen, etc.), but as the core of the science of plant population persistence".

These challenges require the use of AI/ML-DL (machine learning-deep learning), which will enable the detection of stress phenotypes, endpoints in stress effect assessment, image integration, the creation of predictive models of population/species reproductive success, the creation of reference atlases (in embryology: "digital ovule and seed, reproductive digital twin"), and multi-omics.

Research in this area requires interdisciplinarity and a team/consortium including plant embryologists, ecologists, environmental toxicologists, AI/CV (computer vision) specialists, bioinformaticians, genomics, nature restoration and conservation practitioners, seed scientists. Classical embryology, as well as experimental embryology, is the basis for future research (global comparisons). Classical embryology itself can only be a starting point for extensive interdisciplinary research that will help predict the effects of global climate change.

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INSIGHTS FROM SMALL-SPOTED CATSHARK (*SCYLIORHINUS CANICULA*) MYOGENESIS ENHANCE OUR UNDERSTANDING OF THE EVOLUTIONARY PATHWAYS OF MUSCLE DIFFERENTIATION IN FISH

DAMIAN LEWANDOWSKI[1], MAGDA DUBIŃSKA-MAGIERA[1], MARTA MIGOCKA-PATRZAŁEK[1], SYLVIE MAZAN[2], MAŁGORZATA DACZEWSKA[1]

[1]*Department of Animal Developmental Biology, Faculty of Biological Sciences, University of Wroclaw, Sienkiewicza 21, Wroclaw 50-335, Poland*

[2]*CNRS, Sorbonne Universit´e, UMR7232-Biologie Int´egrative des Organismes Marins, Observatoire Oc´eanologique, Banyuls-sur-Mer, France*

Studies on myogenesis in fish have focused on species occupying key phylogenetic positions and have included research on muscle differentiation in chondrosteans, lungfish, and teleosts. Another group of high phylogenetic importance are the cartilaginous fishes. To fill the gap in research on the evolution of muscle differentiation in fish, somitogenesis, muscle differentiation and growth, was investigated in small-spotted catshark (*S. canicula*).

Our investigations revealed that the structure of somites in *S. canicula* shares similarities with lungfish and urodeles, which could reflect plesiomorphic features of small-spotted catshark myogenesis. On the other hand, the myogenesis appear to be apomorphic feature and share numerous features with Osteichthyes and Chondrichthyes representatives, e. g. dermomyotmal origin of muscle progenitor cells or growth of muscle due to PAX3/7-positive cells. Maany issues related to myogenesis in sharks, cartilaginous fish representatives, remain unresolved.

These data provide new insights into myogenesis in Chondrichthyes, the closest outgroup to Osteichthyes, and contribute to a better understanding of ancestral features of this process and its diversification across vertebrates (Lewandowski et al., 2025).

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Exploring non-canonical pathways regulating class II crossovers in *Arabidopsis thaliana*

Agata Lipińska, Franz Boideau, Piotr A. Ziółkowski

Laboratory of Genome Biology, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, ul. Uniwersytetu Poznanskiego 6, 61-614 Poznan, Poland, e-mail: agata.lipinska@amu.edu.pl, franz.boideau@amu.edu.pl, pzio@amu.edu.pl

Faithful processing of meiotic recombination intermediates is essential for accurate chromosome segregation and genome stability. In most eukaryotes including plants, recombination intermediates that involve homologous chromosomes are usually dissolved by DNA helicases (mainly RECQ4 and FANCM), while a small fraction is stabilized by ZMM proteins and repaired as class I crossovers. Intermediates that escape dissolution by helicases and are not protected by ZMM are processed through structure-selective nucleases, including MUS81-EME1, SEND1 and GEN1, which can produce class II crossovers. While MUS81-EME1 defines the major class II crossover (CO) pathway, GEN1 and its paralog SEND1 are proposed backup resolvases. The potential contribution of HIGLE, a plant GIY-YIG endonuclease and putative SLX1 analogue, remains largely unexplored.

The aim of my PhD project is to dissect the genetic relationships within the class II CO pathway and determine how removal of these factors affects meiotic recombination frequency and pathway compensation. I plan to quantify CO frequency in two defined genetic intervals (3.9 and 420) across multiple mutant combinations of *gen1*, *send1*, *higle*, *mus81*, and *fancm*. These analyses will allow me to establish epistatic relationships and test whether GEN1, SEND1, and HIGLE function redundantly with MUS81-EME1 or influence the balance between crossover and non-crossover repair outcomes downstream of FANCM. To further investigate the MUS81 pathway, I have generated a CRISPR/Cas9-induced *eme1b* mutant in an *eme1a* background. I plan to assess recombination frequency in this line to determine how disruption of the MUS81-EME1 nuclease complex affects class II CO formation. For the most informative genotypes, I will perform genotyping-by-sequencing to generate genome-wide crossover maps and evaluate how loss of these nucleases reshapes the crossover landscape, including changes in distribution and potential interference patterns.

Through this work, I aim to clarify the hierarchy, redundancy, and regulatory interactions within the class II recombination pathway and to better understand how auxiliary resolvases shape meiotic crossover formation in plants.

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Dynamics of Cajal bodies' molecular composition as regulators of mRNA retention in *Larix decidua* Mill. microsporocytes

Karolina Majewska, Dariusz Jan Smoliński,
Agnieszka Kołowerzo-Lubnau, Arash Matinahmadi

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland, e-mail: k.majewska@umk.pl

Gene expression in eukaryotic cells is regulated not only at the transcriptional level but also through numerous post-transcriptional mechanisms that enable the cell to flexibly manage the pool of transcripts. One such mechanism is the nuclear retention of poly(A)RNA, which delays the functional use of mRNA. Studies conducted on microsporocytes of European larch (*Larix decidua* Mill.) have demonstrated that mRNA retention is global in nature and remains closely associated with Cajal bodies (CBs) (Rudzka et al., 2022). It has been observed that a significant proportion of transcripts accumulated in CBs contain retained introns, indicating an important role of intron retention in this regulatory mechanism (Rudzka et al., 2022). The aim of studies carried out in recent years has been to assess the scale of this phenomenon, identify transcripts exhibiting intron retention, and analyze changes in the molecular composition of Cajal bodies during the cycle of poly(A) RNA synthesis and export. The obtained results indicate a relationship between the presence of retained introns and the localization of pre-mRNA within CBs, highlighting their importance in the post-transcriptional regulation of gene expression. Furthermore, it has been shown that individual mRNAs differ in their nuclear export dynamics, which may constitute a mechanism for precise temporal control of protein synthesis under conditions of pulsatile transcriptional activity (Rudzka et al., 2022). Cajal bodies also exhibit functional variability during the poly(A) RNA cycle: at early stages, they participate in splicing processes, whereas at later stages, they serve as a reservoir of snRNA used in subsequent cycles of transcriptional activity. These findings therefore indicate a crucial role of Cajal bodies in regulating transcript fate and in controlling processes associated with the development of plant generative cells.

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Unusual multinuclear cells in *Carpetania matritensis* ovary revealed by 3D analysis

Karol Małota, Piotr Świątek

University of Silesia in Katowice, Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, Bankowa 9, 40-007 Katowice, Poland, e-mail: karol.malota@us.edu.pl

Earthworms, or megadriles, are among the most easily recognisable groups of clitellate annelids: usually large, primarily terrestrial, burrowing animals. The largest group of earthworms is Crassiclitellata, comprising over 6,000 species and currently divided into 18 families on all continents except Antarctica. This taxon is regarded as monophyletic; its apomorphy is a multilayered epithelium that forms the clitellum.

In Megadrili, ovaries are paired structures most commonly found in the XIII body segment. Based on data from our previous works (Raś et al., 2025; Świątek et al., 2023), which describe the ovaries as leaf-shaped structures, two major parts can be distinguished: a proximal, broad region attached to the septum, and a distal, narrow region containing growing oocytes. Histological and ultrastructural studies revealed that the typical earthworm ovary can be divided into three zones: Zone I, containing oogonia and early meiotic cells; Zone II, where germ cells enter diplotene; and Zone III, where growing oocytes are found.

Our latest data from 3D analysis of serial ultrathin sections of the *Carpetania martinensis* ovary revealed large, atypical multinucleate cells in the basal part of Zone I. These cells possess electron-lucent cytoplasm and appear to entwine with typical annelid germ cell clusters. Nuage-like material was observed in close proximity to their nuclei. To visualise and better characterise these structures, we prepared 3D reconstructions from Serial Block Face Scanning Electron Microscopy (SBF-SEM). To our knowledge, this is the first description of such cells in earthworm ovaries, and their presence may suggest an involvement in germ cell cluster development. At this early stage of investigation, however, we can only speculate about their precise role in *Carpetania martinensis* oogenesis.

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WHAT'S NEW ABOUT MYOSIN VI IN *DROSOPHILA*? LESSONS FROM MYOSIN VI-DEFICIENT FEMALES

JAKUB OSTROWSKI, KATARZYNA NIEDOJADŁO,
MARTA LENARTOWSKA, ROBERT LENARTOWSKI

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland, e-mail: 503695@doktorant.umk.pl, mlenart@umk.pl, rlenart@umk.pl

Myosin VI (MVI) is a molecular motor that, unlike other myosins, moves toward the minus end of actin filament. The *jaguar* (*jar*) gene, which encodes MVI, was first identified in *Drosophila* embryos, and MVI expression has since been confirmed in other animals, including mammals and humans. This unique protein is involved in many cellular processes, such as actin-based intracellular transport, vesicle trafficking, and actin dynamics. In *Drosophila*, mutations in the *jar* gene were long thought to be lethal, but Morrison and Miller (2008) showed that complete loss of MVI function is not lethal and that the previously reported lethality of the null mutation (jar^{322}) was most likely due to deletion of neighboring genes. However, mutant animals are recovered at a lower than expected Mendelian frequency, suggesting that MVI contributes to normal development. It should be noted that promoter mutations (jar^{1} and jar^{mmw14}) that prevent MVI expression in the testes cause male sterility. So far, MVI deficiency has not been shown to affect female fertility in *Drosophila* (Lenartowski et al., 2025). Our current work provides the first comprehensive characterization of *Drosophila* females lacking MVI, either due to chromosomal deletion or global silencing of MVI expression. We analyze viability and phenotype in both models, focusing on ovarian morphology and oogenesis, including border cell migration.

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SMALL RNA REPERTOIRE OF THE *ARABIDOPSIS THALIANA* EGG CELL

WIKTORIA PARZYCH[1], DAWID BIELEWICZ[2], DAWID KUBIAK[1],
JANUSZ NIEDOJADŁO[1], KATARZYNA NIEDOJADŁO[1],

[1]*Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Torun, Poland, e-mail: wparzych@umk.pl, karask@umk.pl*

[2]*Laboratory of Plant RNA Biology, Center for Advanced Technologies, Adam Mickiewicz University in Poznań, ul. Uniwersytetu Poznańskiego 10, 61-614 Poznań, Poland*

Small non-coding RNAs (sncRNAs) are established regulators of gene expression in plants. The well-characterized classes of 21- and 24-nucleotide microRNAs (miRNAs) and small interfering RNAs (siRNAs) function with ARGONAUTE proteins to mediate transcriptional and post-transcriptional gene silencing, playing central roles in development, stress responses, and reproductive transitions. However, the specific small RNA populations present within the female gamete have remained inaccessible, limiting our understanding of how sncRNA-mediated regulation contributes to gamete identity and the molecular events preceding double fertilization.

The egg cell's unique identity and quiescent state are critical not only for gamete recognition but also for undergoing fertilization and subsequently transitioning to embryogenesis. While the morphological and genetic frameworks of female gametogenesis are increasingly understood in model plants such as *Arabidopsis thaliana*, the post-transcriptional and epigenetic regulatory layers operating within the egg cell itself remain largely unexplored.

To investigate the small RNA landscape of the female gamete, we isolated mature egg cells from *Arabidopsis thaliana* ovules using micromanipulation combined with enzymatic digestion. This method enabled collection of purified egg cells, from which we extracted total RNA and constructed small RNA libraries for deep sequencing. Bioinformatic analysis revealed canonical 21- and 24-nucleotide small RNA classes within the egg cell. Based on this egg cell small RNA transcriptome, we are currently analyzing several candidate miRNAs and siRNAs enriched in the mature egg cell for their potential roles in reproductive fate acquisition and egg cell specification. This dataset provides a foundation for investigating whether previously unreported small noncoding RNAs contribute to the establishment of gamete identity. Ongoing functional characterization of these candidates will offer deeper insight into the epigenetic mechanisms underlying gametophyte development and plant reproduction.

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SERTOLI CELLS IN TELEOST FISH: MORPHOLOGICAL CHANGES DURING SPERMATOGENESIS IN EXTERNALLY AND INTERNALLY FERTILIZING SPECIES

ANNA PECIO[1], ANNA. M. DYMEK[2]

[1]*Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, Kraków 30-387, Poland, e-mail: anna.pecio@uj.edu.pl*

[2]*Department of Small Livestock Breeding, National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland, e-mail: anna.dymek@iz.edu.pl*

Sertoli cells are specialized cells of the germinal epithelium in the testis and play a fundamental role in coordinating spermatogenesis in vertebrates (Sertoli, 1865; Ebner, 1988; Grier 1993; Franca et al., 2015). The use of light microscopy together with transmission (TEM) and scanning (SEM) electron microscopy allows to visualize the interactions between Sertoli cells and germ cells during spermatogenesis in species of Osteoglossiformes, Characiformes and Cyprinodontiformes with external and internal fertilization and releasing free spermatozoa or producing sperm packets.

Results indicate that the ultrastructure of Sertoli cells undergoes continuous remodeling throughout spermatogenesis. At the earliest stages, Sertoli cells enclose a single spermatogonium to form a spermatocyst, which later accommodate by mitotic division to growing number of dozens developing spermatocytes and ultimately to hundreds or thousands spermatids. As spermiogenesis progresses, Sertoli cells assume a phagocytic role, removing residual bodies and degenerating germ cells. After spermiation, they may exhibit bipotentiality and transform into epithelial cells, frequently maintaining phagocytic activity by removing degenerating spermatozoa. In internally fertilizing species, they additionally secrete materials that support the formation of sperm packets called spermatozeugmata or spermatophores.

Our findings highlight profound differences in Sertoli cell ultrastructure and function during reproductive cycle in teleost testes - an organ that uniquely combines the roles of testes, vas deferens, and accessory glands found separately in other vertebrates. These observations underscore the remarkable diversity and plasticity of Sertoli cells in teleost reproductive cycle.

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What is the function of chromatubules in the nuclei of *Utricularia*?

Bartosz J. Płachno[1], Agnieszka Kołowerzo-Lubnau[2], Dariusz Smoliński[2], Katarzyna Niedojadło[2], Piotr Świątek[3], Karol Małota[3], Izabela Kozak[1]

[1]*Institute of Botany, Faculty of Biology Jagiellonian University, 9 Gronostajowa St., 30-387 Cracow, Poland, e-mail: bartosz.plachno@uj.edu.pl; izabela.1.kozak@student.uj.edu.pl*

[2]*Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Torun, Poland, e-mail: agakol@umk.pl; darsmol@umk.pl; karask@umk.pl*

[3]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, 9 Bankowa St., 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl; karol.malota@us.edu.pl*

In the placental cells of *Utricularia nelumbifolia*, unusual nuclei were observed, characterized by distinct protrusions containing paracrystalline structures. These protrusions were connected to the plasma membrane and, *via* the endoplasmic reticulum, to plasmodesmata. These extensions were termed "chromatubules" (tubular structures containing chromatin) (Płachno et al., 2017). Structurally, they partially resemble plastid stromules. These protrusions may serve as pathways for the transport of macromolecules from the nucleus to the plasma membrane and plasmodesmata. However, this hypothesis requires experimental verification. In our study, we investigated the transcriptional activity of cell nuclei with chromatubules in *Utricularia nelumbifolia* and its hybrid (*Utricularia nelumbifolia* × *Utricularia reniformis*). The presence of chromatubules in the hybrid suggests that they are inherited through the maternal line.

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Environmental disinfection strategies in ART laboratories as a critical factor influencing reproductive competence and embryo viability

Wojciech Poćwiardowski[1,2], Agnieszka Bugno[2]

[1]*Department of Chemical Technology and Engineering, Bydgoszcz University of Science and Technology, Seminaryjna 3, 85-326 Bydgoszcz, Poland, e-mail: wojciech.pocwiardowski@pbs.edu.pl*

[2]*Omago Sp. z o.o., Forteczna 13 street, 87-100 Toruń, Poland, e-mail: rd@omago.co*

The controlled laboratory environment in assisted reproduction technologies (ART) is a key determinant of gamete quality, embryo development and reproductive competence (Morbeck, 2015; Wale & Gardner, 2016). Chemical disinfection ensures microbiological safety but may introduce chemical stress affecting sensitive biological structures (ESHRE, 2023).

A review of disinfectants used in ART laboratories was conducted. Alcohols and aldehydes were excluded due to volatile organic compound emissions and their negative impact on embryo development.

Quaternary ammonium compounds (QACs) were evaluated due to their widespread use. Their mechanism involves disruption of microbial membranes and protein denaturation (Gerba, 2015). Although effective against many bacteria, QACs show limited efficacy against spores and non-enveloped viruses, and prolonged exposure may promote resistance. Experimental formulations showed strong antimicrobial activity; however, significant cytotoxicity was observed in ISO 10993-5 assays despite acceptable MEA results (FDA, 2021). This indicates potential embryotoxic risk.

These findings confirm that QAC-based disinfectants require strict protocol adherence. Even at working concentrations, QACs demonstrated limited safety margins, making them a potential risk factor for embryo viability.

Hydrogen peroxide (H_2O_2) exhibits broad antimicrobial activity via reactive oxygen species. However, effective use requires higher concentrations and longer exposure, increasing cytotoxic risk.

The most favorable profile was observed for hypochlorous acid (HOCl). It demonstrates rapid, broad-spectrum antimicrobial activity and, at approximately 200 ppm, shows no embryotoxicity (Block & Rowan, 2020). HOCl does not generate VOCs and degrades into non-toxic by-products, aligning with ESHRE recommendations (ESHRE, 2023).

However, HOCl effectiveness depends on stability. Non-stabilized formulations degrade rapidly, reducing antimicrobial efficacy.

Therefore, only stabilized HOCl formulations with verified concentration and stability should be used in IVF laboratories.

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Germ cell cluster organisation and oogenesis of eutardigrades

Izabela Poprawa[1], Yelyzaveta Matsko[1,2], Marta Jezierska[1], Anna Urbisz[1], Filip Wieczorkiewicz[1,2], Aleksandra Miernik[1,2], Anna Krakowska[1], Karol Małota[1], Kamil Janelt[3]

[1]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, Katowice 40-007, Poland, e-mail: izabela.poprawa@us.edu.pl; anna.urbisz@us.edu.p; anna_krakowska@onet.eu; karol.malota@us.edu.pl*

[2]*Doctoral School, University of Silesia in Katowice, Bankowa 14, 40-007 Katowice, Poland e-mail: yelyzaveta.matsko@us.edu.pl; filip.wieczorkiewicz@us.edu.pl; aleksandra.mierkik@us.edu.pl*

[3]*Department of Medical Genetics, Faculty of Medical Sciences in Katowice, Medical University of Silesia, Medyków 18, Katowice 40-752, Poland, e-mail: kamil.janelt@sum.edu.pl*

Tardigrades belonging to the class Eutardigrada are small invertebrates with a smooth cuticle, devoid of plates and processes. The class Eutardigrada has been divided into two orders: Parachela and Apochela. Tardigrades include gonochoric, hermaphroditic, and parthenogenetic species. Regardless of their reproductive mode, they possess a meroistic ovary, meaning that female germ cell clusters form during oogenesis. Studies using transmission electron microscopy, serial block-face scanning electron microscopy, and 3D reconstruction have allowed the distinction of two types of female germ cell clusters in Eutardigrada. In Parachela, a branching cluster occurs, within which one cell differentiates into an oocyte, while the remaining cells become trophocytes that support the oocyte's development. In Apochela, the cluster consists of several (4-5) multinuclear cells connected by cytoplasmic bridges and numerous mononucleated cells connected to multinuclear cells by cytoplasmic bridges. Within such a cluster, several cells differentiate into oocytes. The multinuclear cells and the remaining mononuclear cells likely function as trophocytes. In both Parachela and Apochela, vitellogenesis is mixed. Yolk is produced partly by autosynthesis in the oocyte and partly by trophocytes. In certain species, structures outside the ovary (storage cells or midgut digestive cells) may also be involved in yolk synthesis. The process of choriogenesis begins during mid- to late-vitellogenesis. The first eggshell to be synthesised is the three-layer chorion. Chorion precursors are synthesised by the oocyte and by the cells of the gonad wall. After chorion formation, the oocyte secretes material for the vitelline envelope. Eutardigrades lay smooth or ornamented eggs. Typically, smooth eggs are laid in the exuvium, while ornamented eggs are laid directly into the environment.

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Organization of earthworm ovaries (Annelida, Crassiclitellata) – from morphology to ultrastructure

Dominika Raś, Piotr Świątek

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland

Earthworms (Megadrili) are terrestrial annelids that function as hermaphrodites with both female and male gonads. Thus, they reproduce by cross-fertilization, but parthenogenetic species also occur. Earthworms' ovaries are paired organs located usually in the XIII segment, and so far, only their localization and general morphology are well-known. Therefore, this research is part of a broader project that aims to describe the general morphology and internal organization of ovaries. We used light and electron microscopy to analyze specimens from different earthworm families. So far, results from the analyzed families (Hormogastridae, Lumbricidae, Megascolecidae, and Eudrilidae) show that ovarian organization differs between families, yet remains conserved at the family level. However, some types of ovarian organization occur in more than one family. Particular types of ovaries can differ in overall shape and in the presence and number of egg strings. Conical-like ovaries with one egg string are observed in Hormogastridae and Lumbricidae families, whereas fan- to rosette-shaped ovaries with numerous radiating egg strings are characteristic of Megascolecidae and Acanthodrilidae families. A much more complex organization was observed in the representative of the family Eudrilidae, where oogenesis was found in three localizations. Despite differences in overall morphology, the internal organization of earthworm ovaries appears identical. Ultrastructural analysis revealed the occurrence of germline cysts (clusters) that interconnect oogonia, early meiotic cells, and nurse cells. Vitellogenic oocytes connected to the cyst have never been observed. Clustering cells are connected by stable intercellular bridges to the poorly developed central cytoplasmic mass – reticular cytophore. Germline cysts equipped with a reticular cytophore were observed in all analyzed families, suggesting that their presence is a conservative feature of earthworm oogenesis.

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Myosin VI and the ultrastructure of mouse epididymal epithelium: apical microvilli and cytoplasmic architecture

Anna Richert[1], Katarzyna Niedojadło[1], Maria Jolanta Rędowicz[2], Marta Lenartowska[1]

[1]*Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland, e-mail: 503436@doktorant.umk.pl*

[2]*Laboratory of Molecular Basis of Cell Motility, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, 02-093 Warsaw, Poland, e-mail: j.redowicz@nencki.edu.pl*

Myosin VI (MYO6) is a unique minus-end-directed actin motor that links the actin cytoskeleton with membrane compartments. In polarized epithelial cells, it plays a dual role by mediating endocytic and recycling pathways and anchoring the apical plasma membrane to the actin core of microvilli. Loss of MYO6 function in mutant mice leads to severe defects, including deafness due to cochlear sensory epithelial dysfunction, impaired nutrient absorption in the intestinal epithelium, and defective renal reabsorption in proximal tubules. In *Drosophila*, mutations in the *MYO6* gene cause male infertility, whereas MYO6-deficient Snell Waltzer mice males exhibit multiple abnormalities during spermiogenesis, accompanied by an approximately 25-30% reduction in fertility. However, sperm counts remain largely unaffected, suggesting that reduced fertility of Snell Waltzer males may primarily result from post-testicular defects. Epididymis is a highly specialized, segmented organ essential for post-testicular sperm maturation and storage. Its diverse epithelial cell types, which are highly active in endocytosis and secretion, establish a luminal microenvironment that supports these events. Our previous work demonstrated that MYO6 accumulates primarily in the caput region, compared with other epididymal segments. Using stimulated emission depletion (STED) super-resolution microscopy, transmission electron microscopy (TEM), and scanning electron microscopy (SEM), we analyzed the ultrastructure of the epididymal epithelium in the caput region of MYO6-deficient mice and identified multiple defects in both principal and clear cells. These included irregular brush border morphology, defective anchoring of microvilli to the apical membrane, partial microvillar loss, disruption of the characteristic two-zone cytoplasmic organization, and abnormal apical bleb formation in subsets of epithelial cells compared with control mice. Our findings demonstrate that MYO6 is essential for maintaining epithelial architecture and apical specialization. Thus, we propose that MYO6 plays a crucial role in the organization and proper functioning of the mouse epididymal epithelium, which is essential for sperm maturation and storage.

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CAN *GALLERIA MELLONELLA* (LEPIDOPTERA, PYRALIDAE) LARVAE BE SAFELY USED IN THE BIODEGRADATION OF PLASTICS?

MAGDALENA ROST-ROSZKOWSKA[1], PATRYCJA MERMER[1], GRAŻYNA WILCZEK[1], JOANNA HOMA[2], ŁUKASZ CHAJEC[1], ANNA WROŃSKA[3], SEBASTIAN STUDENT[4],

[1]*University of Silesia in Katowice, Katowice, Poland,*
e-mail: magdalena.rost-roszkowska@us.edu.pl

[2]*Jagiellonian University, Krakow, Poland*

[3]*Polish Academy of Sciences, Warsaw, Poland*

[4]*Silesian University of Technology, Gliwice, Poland*

Plastics are synthetic polymers whose properties make them widely used in numerous industrial fields, and their global production reaches approximately 350 million tons annually. Plastics undergo spontaneous degradation that can last up to 1000 years. Environmental degradation of plastics can be caused by abiotic factors (UV, temperature, etc.) or by biotic factors (animals and microorganisms present in the environment or in the digestive tracts of animals). Recent studies suggest that over 23 insect species across six orders have been reported to degrade plastics. The greater wax moth, *Galleria mellonella*, is a ubiquitous pest of honey bee (e.g., *Apis mellifera*) hives and causes galleriosis, leading to the colony escaping from the nest. The insect's development includes an egg, seven larval stages, a pupa, and an adult. However, the larvae of *G. mellonella* can feed on various types of plastic, including polypropylene (PP). The aim of our study was to determine if feeding on PP can affect organs at different levels of the animal's body organization. Thus, selected cytotoxicity parameters have been analyzed using TEM, a flow cytometer, and confocal microscopy. The last larval stage (L7) of *G. mellonella* lasts about 7–8 days. Larvae were collected on the 3rd day of the L7 stage and were divided into experimental groups: G0-C – control group, animals fed *ad libitum*; G0-S – animals starved for 24h; G0-24 and G0-48 – animals bred in laboratory conditions (30 ± 0.5°C, constant darkness), previously starved for 24h and then cultured in Petri dishes with pieces of PP bags measuring 3 cm × 3 cm for 24h and 48h respectively. Analysis of the relationship between different cytotoxicity parameters in *G. mellonella* organs (midgut, silk gland, fat body) revealed that the consumption and digestion of PP by larvae do not affect the activation of degenerative processes. Autophagy, DNA synthesis, and GSH are involved in the survival pathway to protect organ cells from the degradation products of plastic. The results of our studies suggest that *G. mellonella* may be considered as a potential candidate for the biodegradation of PP.

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The role of somatic cells in the structure and function of the testes in Anura

Beata Rozenblut-Kościsty[1], Magdalena Chmielewska[1], Katarzyna Haczkiewicz-Leśniak[2], Maria Ogielska[1]

[1]*Department of Evolutionary Biology and Conservation of Vertebrates, Faculty of Biological Sciences, University of Wrocław, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: beata.rozenblut-koscisty@uwr.edu.pl*

[2]*Division of Ultrastructural Research, Wrocław Medical University, Chałubińskiego 6a, 50-368 Wrocław, Poland*

The testis is a heterogeneous organ composed of various cell and tissue types of different origins (Roco et. al., 2021). Although the structure and development of anuran testes are well characterized, most studies have focused on germ cell differentiation, whereas the origins and roles of specific somatic cell lineages have received considerably less attention.

In anurans, germ cells originate from a small group of vegetal blastomeres enriched in maternally derived germ plasm. These cells form primordial germ cells (PGCs), which move passively with the endoderm to the archenteron and then actively migrate to the gonadal primordia (Wylie 1980). After reaching the gonadal ridges, PGCs undergo vitellolysis and differentiate into gonocytes, present only during prespermatogenesis in larval and juvenile stages. Following several mitotic divisions, gonocytes enter quiescence in juvenile males and later, at sexual maturity, differentiate into spermatogonial stem cells (SSCs). Germline cells are structurally and metabolically supported by a variety of somatic cells that form the testicular microenvironment essential for their survival and differentiation.

Somatic cells of the testis originate from several sources.

1. Coelomic epithelium (mesothelium): This epithelium proliferates into the gonad and differentiates into Sertoli cells and peritubular myoid cells. It also gives rise to medullary cells that subsequently form the rete testis.
2. Mesonephric mesenchyme: Mesenchymal cells migrating from the mesonephros differentiate into Leydig cells, fibroblasts, and structural elements that support the rete testis.
3. The fat body: A specialized organ composed of adipocytes, which develops from PGCs located in the most proximal region of the differentiating gonadal primordium.
4. Melanophores: Melanin-containing cells present in the testes of some species. These cells originate from the neural crest, acquire mesenchymal properties during development, and migrate into the gonad.

Together, these endodermal (PGCs), mesodermal (coelomic epithelium and mesonephric mesenchyme), and ectodermal (neural crest) components give rise to the functional gonad. Proper testis activity further depends on its integration with the organism via vascularization and innervation, which support both growth and reproductive function.

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DNA METHYLATION AND HISTONE MODIFICATION PATTERNS DURING ZYGOTIC EMBRYOGENESIS IN HETEROSTYLOUS (*FAGOPYRUM ESCULENTUM*) AND HOMOSTYLOUS (*F. TATARICUM*) BUCKWHEAT

KATARZYNA SALA-CHOLEWA, ALICJA TOMASIAK, KATARZYNA NOWAK, ARTUR PIŃSKI, NATALIA SZKARADEK, ALEXANDER BETEKHTIN

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, 28 Jagiellonska St, Katowice, 40-032, Poland, e-mail: katarzyna.sala@us.edu.pl; alexander.betekhtin@us.edu.pl

Buckwheat is a dicotyledonous plant belonging to the genus *Fagopyrum* (family Polygonaceae), which comprises 22 species; however, only *Fagopyrum esculentum* and *Fagopyrum tataricum* are widely cultivated (Tomasiak et al., 2022). *F. esculentum* is an obligately cross-pollinating, heterostylous species characterized by the presence of two floral morphs (Pin and Thrum), whereas *F. tataricum* is self-pollinating and homostylous (Cavoy et al., 2006). In *F. esculentum*, heterostylous self-incompatibility is associated with pronounced differences in floral morphology, including variation in style and stamen length, pollen size, and intramorph incompatibility. These features contribute to yield instability and pose significant challenges for breeding programs. Although numerous mechanisms related to epigenetic modifications in vegetative plant tissues have been extensively investigated, knowledge regarding DNA methylation and histone modifications during zygotic embryogenesis - particularly in species exhibiting floral dimorphism - remains limited.

The present study characterizes similarities and differences in DNA methylation levels among floral organs of *F. esculentum* involved in pollination and embryo formation, as well as at selected stages of zygotic embryogenesis following pollination in the Pin and Thrum morphs of *F. esculentum*. These patterns are compared with those observed in the self-compatible species *F. tataricum*. Additionally, changes in histone modification levels - specifically histone H3 methylation and histone H3 and H4 acetylation - between the torpedo and cotyledonary stages are analyzed.

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INTER-STRAIN CHIMERIC MICE (*MUS MUSCULUS*) REVEAL THE ROLE OF FETAL ASTROGLIA IN THE PATHOGENESIS OF CORPUS CALLOSUM AGENESIS

SILVESTRE SAMPINO[1], AGNIESZKA BERNAT[1,2], JOANNA CZYRSKA[1], DOMINIKA ŻBIKOWSKA[1], MARIA PIA VISCOMI[1], ABDUL MAJID KHAN[1], DAWID WINIARCZYK[1], MARTA MARLENA ZIĘTEK[1]

[1]*Department of Experimental Embryology, Institute of Genetics and Animal Biotechnology PAN, ul Postępu 36A, 05-552 Jastrzębiec, Poland, e-mails: s.sampino@igbzpan.pl, j.czyrska@igbzpan.pl, d.zbikowska@igbzpan.pl, m.piaviscomi@igbzpan.pl, a.khan@igbzpan.pl, d.winiarczyk@igbzpan.pl, m.zietek@igbzpan.pl*

[2]*Laboratory of Photobiology and Molecular Diagnostics, Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk, ul. Abrahama 58, 80-307 Gdańsk, Poland, e-mail: a.bernat@igbzpan.pl*

The corpus callosum (CC) is the major axon tract of the eutherian brain, connecting and integrating inter-hemispheric neural activities. Developmental defects leading to CC dysgenesis or agenesis affect 1:4000 children worldwide (Paul et al., 2007); however, their etiology and pathogenesis remain poorly understood. To gain insight into the developmental mechanisms underlying CC agenesis, we generated reciprocal inter-strain chimeras by injecting embryonic stem cells (ESC) of the BTBR $T^{+}Itpr3^{tf}$/J (BTBR) mouse model of CC agenesis (Wahlsten et al., 2003) into C57BL/6J (B6) host embryos, and vice versa. BTBR and B6 ESC were first engineered to express a tau-GFP transgene, and subsequently injected into preimplantation embryos, which were then transferred to recipients to generate chimeric mice. This approach allowed tracing axonal projections, including the CC, in the brains of BTBR<->B6 chimeras. We found that ESC of either strain engraft into the host embryo of the other strain and contribute stochastically to multiple brain areas, neuronal circuits, and non-neuronal brain tissues. The CC was present in the brain of 80% of chimeras obtained from BTBR ESC engrafted into B6 host, and in 15% of chimeras obtained from B6 ESC engrafted into BTBR host. Remarkably, BTBR ESC-derived cortical neurons developed callosal inter-hemispheric projections when engrafted in a B6 host, demonstrating the intrinsic competence of BTBR neurons for midline crossing. Since midline astroglial populations, including the midline zipper glia and indusium griseum, are known to guide callosal axons across the interhemispheric fissure (Czyrska et al., 2025), we examined the strain origin of midline astroglia in chimeric brains using GLAST and GFP co-localization by immunostaining. In chimeras where the CC formed, GLAST-positive midline astroglia were predominantly of B6 origin, whereas in chimeras lacking a CC, midline astroglia were predominantly BTBR-derived and displayed a disorganized glial architecture at the interhemispheric fissure. These preliminary observations suggest that fetal midline astroglia is a critical non-neuronal determinant of callosal midline crossing. Moreover, this study supports the use of inter-strain chimeric mice to dissect cell-autonomous from non-cell-autonomous mechanisms in brain developmental defects.

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EXPRESSION OF A- AND B-CONNEXINS IN ORTHO- AND PARAKERATINIZED LINGUAL EPITHELIUM DURING EMBRYONIC DEVELOPMENT OF THE DOMESTIC GOOSE (*ANSER ANSER F. DOMESTICA*) - IHC STUDY

KINGA SKIERESZ-SZEWCZYK, HANNA JACKOWIAK

Department of Anatomy, Histology and Embryology of Animals,
Faculty of Veterinary Medicine and Animal Science, Poznan University of Life Sciences,
Wojska Polskiego 71C, 60-625 Poznań, Poland, e-mail: kinga.skieresz-szewczyk@up.poznan.pl

Keratinocyte differentiation during cornification, along with their proliferation and migration, occurs synchronously due to numerous gap junctions composed of α- and β-connexins (Salomon et al., 1994). In ortho- and parakeratinized epithelium of avian tongue, these junctions include α-connexins (Cx40, Cx43) and β-connexins (Cx26, Cx30, Cx31), from which Cx40, Cx43, and Cx30 facilitate intercellular communication and cornification progression (Skieresz-Szewczyk and Jacowiak, 2023).

The aim of study was to analyze expression profile of α- and β-connexins during histodifferentiation of ortho- and parakeratinized epithelium in developing tongue of domestic goose. Tongues from embryos between days 9^{th} and 25^{th} of incubation were examined using immunohistochemistry for Cx40, Cx43, Cx26, Cx30, and Cx31.

At embryonic stage (9^{th} - $13/14^{th}$ day), medium to strong expression of all connexins was observed throughout undifferentiated epithelia, except for Cx40. During transformation stage ($14/15^{th}$ - $20^{th}/22^{nd}$ day), following formation of basal, intermediate, and superficial layers, expression generally decreased, with medium staining of Cx30 in orthokeratinized epithelium and Cx43 in parakeratinized epithelium. After periderm formation, a primary protective layer, connexin expression increased in upper intermediate layer, except for Cx40. This study shows for the first time that α- and β-connexins are present in periderm, mainly in superficial cells, with weak expression. In pre-hatching stage (23^{rd} - 25^{th} day), when cornified layer forms, expression in lower layers of both epithelia remained unchanged but weaker than in adults. The connexins expression in cornified layer resembled adult patterns in orthokeratinized epithelium, whereas it showed stronger expression in parakeratinized epithelium.

To sum up, Cx43, Cx26, Cx30, and Cx31 are involved in coordination of histodifferentiation of embryonic epithelium into a multilayered epithelium, whereas Cx43 and Cx30 play key roles in initiating cornification of both types of epithelia during embryonic period.

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MITOPHAGY AS A KEY MECHANISM FOR MITOCHONDRIAL QUALITY CONTROL IN THE OOCYTES OF THE BUSH-CRICKET *ROESELIANA ROESELII* (ORTHOPTERA: TETTIGONIIDAE)

MAGDA SOCHACZEWSKA[1,2], WACLAW TWORZYDLO[1]

[1]*Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Lojasiewicza 11, 30-348 Krakow, Poland, e-mail: magda.sochaczewska@doctoral.uj.edu.pl*

One explanation of cellular ageing involves a significant decline in mitochondrial activity. According to this concept, the integrity and functionality of mitochondrial DNA (mtDNA) deteriorate over time due to the gradual accumulation of harmful mutations. In most animals, mitochondria are inherited exclusively through the maternal line and are derived from the female germline cells (oocytes) only. Due to the lack of genetic recombination, mtDNA would quickly accumulate deleterious mutations. Therefore, the precise mechanisms must exist in the female germ cells to limit the transmission of damaged mtDNA copies.

In this study, previtellogenic oocytes of the bush-cricket *Roeseliana roeselii* were analyzed across larval, adult, and old females using a combination of morphological, histochemical, and immunocytochemical techniques. Ultrastructural analyses revealed the presence of variable in size membrane-bound structures, frequently containing material with high electron density. Among these materials, mitochondria have been observed. These structures are distributed throughout the ooplasm, with the highest abundance typically observed in the perinuclear region. Notably, such compartments were detected at all examined developmental stages of bush-cricket.

Our histochemical analyses demonstrated colocalization of mitochondria and lysosomes, suggesting that the observed membrane-bound structures are associated with mitophagy. To further support this interpretation, immunocytochemical analyses targeting autophagy-related proteins, such as Beclin-1, Atg5, LC3, LAMP1 and ubiquitin, a marker of autophagic targeting, were performed. The obtained results demonstrate that these proteins are frequently colocalized with mitochondria. Importantly, this pattern was consistently observed across oocytes derived from larvae, adult, and old females.

Based on these findings, we interpret the membrane-associated compartments as autophagolysosomes and propose that mitophagy plays a pivotal role in ensuring the quality of mitochondria transmitted between generations.

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KNOCKDOWN OF *CALNEXIN1* DISRUPTS POLLEN TUBE TIP GROWTH IN *PETUNIA*

ANNA SUWIŃSKA, ROBERT LENARTOWSKI, MARTA LENARTOWSKA

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Torun, Poland, e-mail: asu@umk.pl

The pollen tube of flowering plants represents one of the fastest-growing plant cell types. Its polarized tip growth requires precise coordination of key cellular processes, including maintenance of the Ca^{2+} gradient, organization of internal membrane systems, and cytoskeleton dynamics. Sustaining a high growth rate is also associated with intensive protein synthesis, maturation, and secretion. A key role in regulating these processes is played by the endoplasmic reticulum (ER), where newly synthesized proteins undergo folding with the assistance of molecular chaperones. Our previous studies in *Petunia hybrida* demonstrated that calreticulin (CRT), a Ca^{2+}-binding luminal ER chaperone, plays an important role in pollen tube growth regulation. Silencing of CRT expression leads to disruption of Ca^{2+} homeostasis, disorganization of the actin cytoskeleton, and inhibition of pollen tube growth (Suwińska et al., 2017). Since CRT functionally cooperates with calnexin (CNX), a membrane-bound ER chaperone involved in glycoprotein folding, we hypothesized that CNX may play a similarly important role in these processes. In *Petunia*, two *CNX* homologs, *PhCNX1* and *PhCNX2*, have been cloned and characterized (Suwińska et al., 2025). To verify this hypothesis, we silenced *PhCNX1* gene expression using RNA interference (RNAi) in pollen tubes growing in vitro. Our results showed that depletion of *Ph*CNX1 leads to alterations in pollen tube morphology and impaired growth. At the cellular level, we observed abnormalities in actin cytoskeleton organization as well as disrupted distribution of organelles, vesicles, and cell wall components. These results indicate that CNX, most likely in cooperation with CRT, plays an important role in maintaining the functional integrity of the pollen tube by regulating cellular processes required for its proper tip growth.

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STRATEGIES OF TRANSOVARIAL TRANSMISSION OF SYMBIOTIC MICROORGANISMS IN PLANTHOPPERS (INSECTA, HEMIPTERA, FULGOROMORPHA)

TERESA SZKLARZEWICZ, ANNA MICHALIK

Department of Developmental Biology and Morphology of Invertebrates, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, e-mail: teresa.szklarzewicz@uj.edu.pl

Planthoppers, like other plant sap-sucking hemipterans, are host to symbiotic microorganisms which supplement their restricted diet with missing nutrients. Most planthoppers are accompanied by two symbionts of ancient origin: bacteria *Sulcia* and *Vidania*, which in some species coexist with additional bacteria such as *Sodalis*, *Arsenophonus*, *Purcelliella*, *Wolbachia*, *Rickettsia*, or *Acetobacteraceae*. During planthopper evolution, in some lineages ancestral symbionts have been eliminated and replaced by other bacteria or Hypocreales fungi. As a rule, bacterial symbionts are located in the cytoplasm of own cells termed bacteriocytes, less frequently they share bacteriocytes with other bacteria or occupy fat body cells. Bacteria *Vidania* occur both in bacteriocytes localized in the body cavity and in bacteriocytes lying in the invagination of the hindgut, termed the rectal organ. Fungal symbionts are located in large syncytial organs termed mycetomes.

In planthoppers, both symbiotic bacteria and fungi are transmitted between generations transowarially, i.e. as a result of infection of ovaries. Microscopic observations revealed that planthoppers developed different strategies of symbiont transmission, however, the initiation of ovary infection (i.e. release of bacteria from bacteriocytes/rectal organ, their migration towards the ovaries) is always corelated with the stage of oogenesis. In most planthoppers the transmission process is highly conserved - *Sulcia* and *Vidania* as well as fungi and additional bacterial symbionts invade the posterior pole of ovarioles containing terminal oocytes at the stage of advanced vitellogenesis. Symbionts subsequently: pass through the follicular epithelium (*via* endocytic/exocytic pathway), enter the space between oocyte and follicular epithelium, and gather in the deep invagination of oolemma in the form of "symbiont ball". However, in some planthoppers alternative transmission strategies have evolved, e.g. in *Trypetimorpha occidentalis* (Tropiduchidae), the transmission of symbionts takes similar course as in most planthoppers, but migrating *Acetobacteraceae* symbionts remain hidden in large *Sulcia* cells. In turn, in some Dictyopharidae planthoppers symbiont transmission is separated in time and space - *Sulcia* and *Vidania* infect posterior end of the ovariole containing vitellogenic oocytes, whereas *Sodalis* bacteria invade previtellogenic oocytes occupying the apical region of the vitellarium.

These observations highlight the remarkable diversity and evolutionary flexibility of symbiont transmission strategies in planthoppers, despite the overall conservation of vertical inheritance mechanisms.

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ASPROSIN REGULATES NUCLEAR AND CYTOPLASMIC MATURATION OF MOUSE OOCYTES. *IN VITRO* STUDY

OLIWIA SZKRABA[1,2], PATRYCJA KUROWSKA[1], EDYTA RYTELEWSKA[1], AGNIESZKA RAK[1]

[1]*Laboratory of Physiology and Toxicology of Reproduction, Institute of Zoology and Biomedical Research, Jagiellonian University, Kraków, Poland*

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland*

The development of gametes is influenced by the organism's energy status and hormonal signals. Asprosin is a metabolic hormone secreted by fat tissue that plays a role in regulating blood glucose levels and energy balance. Increasing evidence suggest that hormones and metabolic factors can also affect reproductive processes but the role of asprosin in the maturation of female gametes remains unclear. The aim of this study was to investigate (i) expression of asprosin, its processing enzyme furin and its receptor Olfr734 in mouse oocyte and (ii) asprosin effects on nuclear and cytoplasmic maturation of mouse oocytes during *in vitro* maturation (IVM).

Female C57BL/J mice (8–12 weeks old) were stimulated to superovulate by intraperitoneal injection of 7.5 IU pregnant mare serum gonadotropin (PMSG) 48 h before sacrifice and ovarian collection. Oocytes were isolated from ovaries and cultured for 24 h under control conditions or in the presence of 10 nM asprosin. After maturation (18h), oocytes and culture media were subjected to further analyses. Nuclear maturation was evaluated by DAPI staining to visualize first polar body extrusion. Gene expression of oocyte-derived factors (Bmp15, Gdf9), lipid metabolism markers (Pnpla2, Plin2), and glucose transporter (Scl2a4) were determined using qRT-PCR. Progesterone (P4) and glucose concentrations in post-culture media were measured by ELISA. Lipid droplet accumulation in oocytes was assessed by Nile Red staining. Additionally, expression of asprosin, furin and asprosin's receptor Olfr734 was analyzed in mouse ovarian sections using immunohistochemical staining.

The obtained results showed that 45% of oocytes cultured in the presence of asprosin reached the metaphase II stage and extruded the first polar body ($n = 5$, $p \leq 0.05$). Asprosin treatment significantly increased mRNA levels of Bmp15 and Gdf9, suggesting enhanced oocyte developmental competence ($n = 5$, $p \leq 0.05$). In addition, expression of lipid metabolism-related genes Pnpla2 and Plin2 was elevated in asprosin-treated oocytes ($n = 5$, $p \leq 0.05$). Consistently, Nile Red staining revealed increased lipid droplet accumulation following asprosin exposure ($n = 5$, $p \leq 0.05$). Analysis of culture media demonstrated that asprosin stimulated P4 secretion and increased glucose levels ($n = 5$, $p \leq 0.05$). In contrast, no differences in Scl2a4 mRNA expression were observed between control and asprosin-treated oocytes ($n = 5$, $p \leq 0.05$). Immunohistochemical analysis demonstrated the presence of asprosin, furin, and Olfr734 in the mouse ovary ($n = 5$, $p \leq 0.05$). Asprosin and furin were detected in all ovarian structures, predominantly in granulosa cells, whereas Olfr734 expression was mainly localized in the oocyte ($n = 5$, $p \leq 0.05$).

In conclusion, these findings suggest that asprosin modulates both nuclear and cytoplasmic maturation of mouse oocytes *in vitro*. The stimulatory effects of asprosin on genes related to oocyte quality, lipid metabolism and P4 secretion indicate that this adipokine may participate in metabolic regulation of oocyte maturation.

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Diverse forms of female germline clusters in clitellate annelids

Piotr Świątek, Anna Z. Urbisz

Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland, e-mail: piotr.swiatek@us.edu.pl, anna.urbisz@us.edu.pl

The syncytial cysts (clusters) that interconnect female germline cells at the onset of oogenesis are typical of most metazoans. Oogonia and early meiotic cells are interconnected by relatively wide cytoplasmic connections (intercellular bridges, ring canals) that ensure cytoplasmic continuity between them. The spatial organization of such cysts differs between taxa. In clitellate annelids (Clitellata), both male and female cysts have the same conserved pattern; each cell is connected to the central, anuclear cytoplasmic core (cytophore) via a single ring canal. Thus, the cells are not directly interconnected; the cytophore mediates the transfer of cell components between cells. Such a pattern of germline cyst architecture is found in e.g. nematodes, flatworms, and polychaete annelids, but is absent in e.g., insects or vertebrates.

Despite the conserved architecture, female cysts in clitellates may differ in the number of interconnected cells, in the shape and dimensions of the cytophore, and in the number of developing oocytes. In cysts with a low number of clustering cells (from eight to maximally 60, the so-called oligocellular cysts), the cytophore has a form of thin cytoplasmic stands (the reticular cytophore) or is ball-like. The reticular cytophore interconnecting eight cells is characteristic of earthworms and allied taxa only. The ball-like cytophore unites 16 cells in white worms (e.g., *Enchytraeus albidus*), 32 cells in some naidins and pherodrilids, and around 40-60 cells in fish leeches. When the cysts are composed of hundreds or even thousands of cells (multicellular cysts), the cytophore is huge and tree-like. Such clusters have been found in numerous taxa, e.g., sludge worms (e.g., *Tubifex tubifex*), haplotaxids, and propappids. In *T. tubifex*, cysts may contain over 2,000 cells, and the tree-like cytophore forms numerous "branches" which snake between cells. In oligocellular cysts, usually one cell becomes an oocyte; the rest do not grow and function as nurse cells. In multicellular cysts, several (even a dozen or so) growing oocytes can be found in a given cyst. Multicellular cysts are so large that only one is usually found within an ovary (cyst=ovary); if oligocellular cysts occur, their number per ovary is higher (several or dozens).

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A POSTERIOR POLE LYSOSOMAL COMPARTMENT PRESENT IN OOCYTES OF DERIVED DERMAPTERANS RESEMBLES ENDOLYSOSOMAL VESICULAR ASSEMBLIES DESCRIBED IN MAMMALIAN OOCYTES

Waclaw Tworzydlo[1], Magda Sochaczewska[1,2], Anna Michalik[1], Szczepan Bilinski[1]

[1]*Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Krakow, Poland, e-mail: w.tworzydlo@uj.edu.pl*

Oocytes of studied derived earwigs (*Forficula auricularia* and *Apterygida media*), besides typical organelles such as mitochondria, endoplasmic reticulum, Golgi complexes, lysosome-like bodies, and reserve materials also possess a surprisingly diverse set of noncanonical organelles. These include complexes of endoplasmic reticulum associated with fine granular nuage material, ribosome-associated vesicles (termed RAVs), and extensive arrays of parallel endoplasmic reticulum cisternae. The oocytes of *A. media* additionally contain dozens of symbiotic bacteria. Amplicon sequencing of 16S rDNA revealed that the symbiotic bacteria belong to α-proteobacteria from *Wolbachia* and *Rickettsia* genera.

During vitellogenesis, lysosome-like bodies (and bacteria in *A. media*) progressively relocate and reach the posterior pole of the oocyte, where they assemble into a relatively large and morphologically distinct structure, which we termed the posterior pole lysosomal compartment. This compartment lacks a surrounding limiting membrane and shows positive staining with Proteostat, a dye commonly used to detect proteins involved in biomolecular condensate formation.

Based on these observations, we hypothesize that the posterior pole lysosomal compartment resembles endolysosomal vesicular assemblies in mammalian oocytes that are implicated in the sequestration and degradation of aggregated proteins.

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CALRETICULIN 3A IS ASSOCIATED WITH MALE GAMETOPHYTE DEVELOPMENT IN *PETUNIA HYBRIDA*

PIOTR WASĄG, ANNA SUWIŃSKA, MARTA LENARTOWSKA, ROBERT LENARTOWSKI

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland, e-mail: piwas@umk.pl

In flowering plants, male gamete development occurs within the anther, where pollen grains are formed during successive stages of microsporo/gametogenesis. This complex process involves the differentiation of microspore mother cells, meiosis, and the maturation of haploid microspores into functional pollen. Pollen development is supported by the tapetum, a specialized anther tissue that provides nutrients to developing germline cells and contributes to sporoderm biosynthesis. The formation of the male gametophyte is associated with elevated metabolic activity across anther tissues, particularly in the tapetum, and relies on the functional integrity of the endoplasmic reticulum (ER). ER-resident chaperones play essential roles in protein folding and quality control, as well as in the regulation of calcium homeostasis. Among these, calreticulin (CRT) is a highly conserved calcium-binding protein belonging to the lectin-like reticuloplasmins, implicated in ER quality control and stress responses. In plants, CRTs comprise three functionally distinct isoforms, including the plant-specific CRT3, whose role in male gametophyte development remains poorly understood. In our previous study, we identified *PhCRT3a*, a CRT3 homolog from *Petunia hybrida*, and demonstrated that siRNA-mediated post-transcriptional silencing of this gene in pollen tubes causes severe morphological and ultrastructural defects, resulting in impaired in vitro tube growth (Wasąg et al., 2022). Based on these findings, the present study focuses on the spatiotemporal expression profile of *Ph*CRT3a during male gametophyte formation. Transcript abundance and protein localization were examined at successive stages of pollen formation using RT-qPCR, fluorescence in situ hybridization (FISH), and immunocytochemistry. These analyzes provide new insights into the potential role of CRT3 in the regulation of pollen development.

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Analysis of seed germination barriers of the leafless iris (*Iris aphylla* L.) in the context of species protection

Krystyna Winiarczyk[1], Magdalena Śmigała-Lasota[1], Bożena Denisow[2]

[1]*Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, e-mail: krystyna.winiarczyk@umcs.pl*

[2]*Department of Botany and Plant Physiology, University of Life Sciences in Lublin, 15 Akademicka St., 20-950, Lublin, Poland, e-mail: bozena.denisow@up.lublin.pl*

Iris aphylla is a strictly protected species, classified as vulnerable due to a progressive decline in population numbers. To develop effective *ex situ* conservation and reintroduction strategies, it has become crucial to identify the causes of low generative reproduction efficiency and to analyze the flowering and fruiting biology of this taxon. In the course of this study, attempts were made to break seed dormancy using standard procedures described in the literature, including mechanical and chemical methods. It was observed that conventional germination stimulation techniques are ineffective for the analyzed species. Consequently, *in vitro* isolated embryo culture techniques were implemented. Upon excision from the endosperm, embryos were placed on agar media. Maximum process efficiency was achieved exclusively through embryo isolation and subsequent *in vitro* cultivation. Simultaneously, biochemical analyses of seed structures (embryo, endosperm, and seed coat) were conducted to determine the phytohormonal profile, with *Iris sibirica* seeds serving as the comparative group. The analysis revealed a significant accumulation of salicylic acid in *I. aphylla* seeds (values 8–10 times higher than in *I. sibirica*) and an unfavorable abscisic acid (ABA) to gibberellin (GA) ratio within the endosperm tissue. These results support the hypothesis that the endosperm in *I. aphylla*, beyond its storage function, acts as an active regulatory center that controls seed physiology and induces deep embryo dormancy through hormonal interactions. In conclusion, it was demonstrated that an effective strategy for *ex situ* conservation and species reintroduction is the mechanical isolation of embryos combined with *in vitro* techniques. This procedure allows for the elimination of the inhibitory influence of the endosperm; isolated embryos exhibit full capacity for proper development, enabling the production of plant material ready for translocation to natural sites after a several-month acclimatization period. Additionally, existing reports regarding the impact of the iris weevil (*Mononychus punctumalbum*) on *I. aphylla* population dynamics were verified. Observations indicate that the weevil does not feed on the fruits but destroys the stamens and pistils within the flowers, which constitutes a significant barrier limiting the seed-setting process.

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LIPID DROPLET MOBILIZATION VIA B-OXIDATION REGULATES TROPHECTODERM HOMEOSTASIS IN PREIMPLANTATION MOUSE EMBRYOS (*MUS MUSCULUS*)

DOMINIKA ŻBIKOWSKA[1], ABDUL MAJID KHAN[1], MARTA MARLENA ZIĘTEK[1], DOMENICO IUSO[2], SILVESTRE SAMPINO[1]

[1]*Department of Experimental Embryology, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, ul. Postępu 36A, 05-552, Jastrzębiec, Poland, e-mail: d.zbikowska@igbzpan.pl, a.khan@igbzpan.pl, m.zietek@igbzpan.pl, s.sampino@igbzpan.pl*

[2]*Faculty of Veterinary Sciences, University of Teramo, Via Renato Balzarini 1, 64100 Teramo, Italy, e-mail: diuso@unite.it*

Lipid droplets (LD) in the cytoplasm of preimplantation embryos serve as reservoirs of fatty acids (FA) for mitochondrial β-oxidation. During development, LDs undergo dynamic changes in number, morphology, and distribution, reflecting adaptation to stage-specific metabolic demands. However, it remains unclear whether FA mobilization from LD supports differentiation of the first embryonic lineages. The BTBR $T^{+}Itpr3^{tf}$/J mouse strain, characterized by numerous metabolic disturbances and significantly reduced LD content in zygotes, is an excellent model to study the impact of disrupted LD homeostasis on early preimplantation development.

BTBR and control C57BL/6J (B6) embryos were cultured in KSOM medium with etomoxir (β-oxidation inhibitor), L-carnitine (β-oxidation stimulator), or without additives. LD volume, number and distribution were analyzed by BODIPY staining and confocal microscopy, while lineage-specific localization was assessed by immunostaining for CDX2 (TE) and SOX2 (ICM).

B6 embryos accumulate lipids in large LD throughout the preimplantation period. In contrast, BTBR embryos rapidly mobilize LD at the morula stage, correlating with delayed cavitation and reduced viability. In B6 blastocysts, LD are evenly distributed between TE and ICM, whereas BTBR blastocysts show almost complete depletion of large LD in the TE. Pharmacological interventions confirmed the critical role of β-oxidation in shaping these phenotypes. Etomoxir (25 μM) induced excessive LD accumulation in B6 embryos, while BTBR embryos were largely resistant; only 100 μM etomoxir partially restored LD in BTBR TE. Conversely, L-carnitine strongly promoted LD mobilization in both strains, making B6 embryos resemble the TE lipid profile of BTBR.

Disrupted LD homeostasis impairs preimplantation embryo development. Fatty acid mobilization from LD appears particularly important for proper TE function. These findings reveal a critical link between β-oxidation and lineage-specific metabolic regulation in early mouse embryos.

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Changes in the cell cycle and proliferation in the midgut epithelium of *Lithobius forficatus* (Linnaeus, 1758) (Myriapoda: Chilopoda) induced by various concentrations of nanostyrene

Agata Aleksa, Łukasz Chajec, Anna Ostróżka, Grażyna Wilczek, Magdalena Rost-Roszkowska

University of Silesia in Katowice, Faculty of Natural Sciences, Institute of Biology, Biotechnology and Environmental Protection, Bankowa 9, 40-007 Katowice, Poland, e-mails: agata.aleksa@us.edu.pl, lukasz.chajec@us.edu.pl, anna.ostrozka@us.edu.pl, magdalena.rost-roszkowska@us.edu.pl

Plastic pollution is one of the most serious challenges facing modern ecology. Microplastics degrade into nanoplastics, such as nanostyrene, which can accumulate in the soil and affect soil organisms. Due to their small size, nanoplastics can penetrate cell membranes and accumulate within cells and their organelles, potentially disrupting cellular processes. The aim of this study was to assess the effect of nanostyrene on the cell cycle and proliferative activity of midgut epithelial cells of *Lithobius forficatus*. Adult specimens were reared in soil containing nanostyrene at concentrations of 10 mg and 100 mg/kg dry soil weight for periods of 1, 3, and 6 months, enabling the analysis of the effects of short- and long-term exposure. Flow cytometry was used to assess changes in the cell cycle, determining the proportion of cells in the G0/G1, S, and G2/M phases. Additionally, cell proliferation activity was analysed to assess potential changes in the regenerative processes of the studied epithelium. The results obtained may contribute to a better understanding of the mechanisms by which nanoplastics affect soil organisms, particularly at the cellular level, and provide a basis for further research into their impact on the functioning of soil ecosystems.

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Chromosomal rDNA distribution patterns in *Cobitis* tetraploid hybrids (Teleostei, Cobitidae): insights into parental genomic contributions

Alicja Boroń, Anna Grabowska, Olga Jabłońska, Lech Kirtiklis, Dorota Juchno

Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-959 Olsztyn, Poland, e-mail: alibo@uwm.edu.pl, agrabowska@iimcb.gov.pl, olga.jablonska@uwm.edu.pl, leo@uwm.edu.pl, juchno@uwm.edu.pl

Taxa from the genus *Cobitis* evolving through hybridization and polyploidization represent an interesting model for studying these processes and their role in vertebrate evolution. The study presents cytogenetic data on different hybrid tetraploid *Cobitis* taxa (4n=97–99) from mixed populations dominated by allotriploid females coexisting with *C. taenia* as one of the parental species. Chromosome structure were examined using *in situ* hybridization (FISH) with 5S and 28S rDNA probes. The rDNA patterns indicate certain marker chromosomes enabling identification of the parental species in the hybrid taxa. The 5S rDNA was identified as an effective diagnostic marker of *C. elongatoides* – the second of parental species - allowing partial discrimination of the genomic composition of diploid, triploid as well as tetraploid *Cobitis* hybrids. In the genomes of polyploid *Cobitis* taxa, a set of chromosomes originating from *C. tanaitica*, a species not present in Poland, was detected. The karyotypes of tetraploid *Cobitis* hybrids reflect the genomic contributions of their parental species. The observed numbers of both 28S and 5S rDNA signals in diploid and polyploid *Cobitis* hybrids were disproportionally inherited from the two parental species. The chromosome sets of *C. taenia* in polyploid hybrids contained fewer chromosomes with 5S rDNA loci. The obtained results indicate a differentiated role of particular *Cobitis* species in the formation and maintenance of hybrid lineages. The detected variability in rDNA distribution patterns suggests complex genomic interactions in *Cobitis* hybrids resulting from polyploidization and hybridization, potentially influencing their reproductive potential.

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THE COMPARISON OF CALLUS ACTIVITY AND BIOCHEMICAL COMPOSITION OF *CYNARA CARDUNCULUS* VAR *SCOLYMUS* L. (ASTERACEAE) IN *IN VITRO* AND *IN VIVO* CONDITIONS USING IR SPECTROSCOPY

PIOTR CELUCH, MARCIN DOMACIUK, MARIUSZ GAGOŚ, ALEKSANDRA ŚMIAŁKO

Department of Cell Biology, Faculty of Biology and Biotechnology, University of Maria Curie-Sklodowska in Lublin, Akademicka 19, 20-033 Lublin, Poland, e-mail: piotr.celuch@mail.umcs.pl

The following studies covers the difference between the activity of different types of callus origin. As the callus cultures are one of the most important cell culture. In botany following the somatic embriogenesis, propagation and genetic engenering etc. the understanding of biochemical changes in composition of this type of cell is needed. This studies are part of preliminary studies with usage of Fourier Transform Infrared Spectroscopy. FTIR spectrum analysis allowed for a comparison of the chemical composition of callus obtained from leaves *in vivo* and leaves grown *in vitro*. In the range of 3000–2800 cm^{-1}, corresponding to C–H stretching vibrations in CH_2 and CH_3 groups, higher band intensities were observed for *in vitro* callus. This may indicate a higher content of membrane lipids or their reorganization, which is typical for tissues with high metabolic and proliferative activity. In the 1600–1800 cm^{-1} range of the IR spectrum, characteristic of COO^- and carbonyl group vibrations, clear differences between the samples were observed. The band at 1658 cm^{-1}, associated with C=O vibrations of amides I and aromatic groups, showed a slight batochromic shift in tissues cultured *in vitro*. This change suggests enhanced hydrogen bonding and modifications in the chemical environment of proteins. Additionally, the higher intensity of amide bands in *in vitro* cultures indicates more intense protein synthesis, which can be associated with dynamic cell division processes and metabolic remodeling. In the range of 1550–1515 cm^{-1}, the bands associated with pectins and lignin showed higher intensity in *in vitro* tissues. This may suggest active cell wall remodeling and the participation of lignin-like polymers in adaptation to artificial culture conditions. In the 1200–900 cm^{-1} region, differences were observed, particularly around 1050 cm^{-1}, corresponding to C–O and C–O–C vibrations in cellulose and hemicelluloses. In *in vivo* tissues, these signals were stronger which indicates the presence of a well-developed polysaccharide structure of the cell wall characteristic of leaves. In *in vitro* callus, the intensity of these bands was lower, suggesting more flexible cell wall structure typical of undifferentiated callus cells.

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SILENCING OF MYOSIN VI GENE EXPRESSION IN BORDER CELLS – IMMUNOCYTOCHEMICAL STUDIES AND ANALYSIS OF ACTIN CYTOSKELETON IN *DROSOPHILA* OOGENESIS

ALEKSANDRA CHROBOT, JAKUB OSTROWSKI, MARTA LENARTOWSKA

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Torun, Poland, e-mails: 312468@stud.umk.pl, 503695@doktorant.umk.pl, mlenart@umk.pl

Oogenesis in *Drosophila melanogaster* is a complex process involving numerous morphological changes and dynamic reorganization of the cytoskeleton in developing egg chambers. A particularly important process is the collective migration of border cells (BCs), which move as a cluster toward the oocyte of the maturing egg chamber. The proper progression of these events requires the involvement of proteins interacting with the actin cytoskeleton, including motor proteins such as myosins (Żłobiński et al., 2013). Among myosins, only myosin VI (MVI) has the ability to move toward the minus end of the actin filaments (Lenartowski et al., 2025).

The aim of this study was to analyze the role of this unique molecular motor, myosin VI, during oogenesis in *Drosophila* after silencing its expression specifically in border cells. By using immunocytochemical labeling of MVI and phalloidin staining, comparative characterization of egg chambers development in individuals with silenced MVI expression in BCs relative to control, was performed. The successive stages of border cell movement - recruitment, collective migration, and reaching the oocyte - were particularly analyzed, along with the distribution of actin filaments. The results provide new insights into the role of myosin VI in *Drosophila* oogenesis and the effects of its silencing on the actin cytoskeleton.

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UNRAVELING DEVELOPMENTAL DELAY IN HAPLOID OAT (*AVENA SATIVA* L.) EMBRYOS: ANATOMICAL AND METABOLIC INSIGHTS

KINGA DZIURKA[1], MICHAŁ DZIURKA[1], EWA MUSZYŃSKA[2]

[1]*Department of Biotechnology, The F. Górski Institute of Plant Physiology Polish Academy of Sciences, 30-239 Kraków, Niezapominajek 21, Poland, e-mails: k.dziurka@ifr-pan.edu.pl, michal.dziurka@gmail.com*

[2]*Department of Botany and Plant Physiology, Institute of Biology, Warsaw University of Life Sciences-SGGW, 02-776 Warsaw, Nowoursynowska 159, Poland, e-mail: ewa_muszynska@sggw.edu.pl*

While doubled haploid production is a cornerstone of modern oat (*Avena sativa* L.) breeding, its efficiency is severely limited by the developmental arrest of haploid embryos. Therefore, 21-day-old haploid oat embryos obtained through wide crossing with maize (*Zea mays* L.) were subjected to detailed analyses. The research employed an interdisciplinary approach, combining microscopic imaging methods with advanced metabolic profiling using the HPLC-MS/MS technique.

It was found that morphologically and anatomically haploid embryos corresponded to zygotic embryos at a stage earlier than 9 DAP (days after pollination) (Dziurka et al., 2025a). In turn, ultrastructural examination confirmed the presence of starch grains and lipid droplets, indicating the accumulation of reserve materials in embryonic cells. Biochemical analysis revealed that sucrose was the dominant sugar, followed by raffinose, glucose, and fructose at three-fold lower concentrations (Dziurka et al., 2025b). The haploid embryos were also characterized by a high protein content, averaging approximately 150 µg/mg. Among the amino acids, methionine accumulated in the highest amounts (approx. 80 µg/g DW) (Dziurka et al., 2025b), while asparagine and proline were also significantly represented. Unlike zygotic embryos, haploid ones accumulated primarily auxin conjugates (IAA-Asp, IAA-Glu, meIAA) rather than the free (active) form of IAA (Dziurka et al., 2025a). Additionally, the phenolic profile of haploids was dominated by syringic acid, alongside notable quantities of benzoic, gallic, chlorogenic, and caffeic acids. The conducted experiments clearly demonstrate that haploid embryos, although anatomically immature, possess an extensive metabolic apparatus and a distinct hormonal regulatory system.

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LOCALIZATION OF CNX1 PROTEIN IN THE STIGMA OF *PETUNIA*

OLIWIA GOŁĘBIEWSKA, ANNA SUWIŃSKA

Department of Cellular and Molecular Biology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Lwowska 1, 87-100 Torun, Poland, e-mail: 312471@stud.umk.pl

Successful fertilization in angiosperms depends on precise interactions between pollen grains and the stigma. It is on the stigma that pollen recognition, hydration, germination, and subsequent pollen tube growth occur, enabling the transport of sperm cells to the embryo sac for double fertilization. Both stigma cells and rapidly growing pollen tubes require efficient protein synthesis and proper folding of newly synthesized proteins. A key role in these processes is played by the endoplasmic reticulum (ER), which hosts a protein quality control system based on molecular chaperones. One of these is calnexin (CNX), an integral ER protein that, together with calreticulin (CRT), participates in the CNX/CRT cycle, ensuring proper folding and quality control of newly synthesized glycoproteins (Kozlov and Gehring, 2020). In plants, CNX is involved in multiple physiological processes, including growth, development, and responses to abiotic stress. However, its specific function in reproductive processes remains poorly understood. In our previous studies, we cloned and characterized two *CNX* homologs in *Petunia hybrida* (*PhCNX1* and *PhCNX2*) and demonstrated that their expression is dynamically regulated during microsporogenesis as well as during pollen germination and pollen tube growth in vitro (Suwińska et al., 2025). Based on these observations, we hypothesize that CNX may also play an important role in protein maturation processes occurring in the stigma during pollination. In the present study, we demonstrate the presence of CNX1 protein in the stigma of *Petunia*. Using immunocytochemistry, we showed that prior to pollination CNX1 is predominantly localized in secretory stigma cells. Following pollination, CNX1 accumulates in hydrated and germinating pollen grains as well as in elongating pollen tubes, accompanied by a decrease in signal within stigma cells. These results indicate that CNX1 may play an important role in glycoprotein folding and quality control both in sporophytic stigma tissues and in the male gametophyte, highlighting its involvement in the regulation of secretory processes associated with pollination and pollen tube growth.

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INDUCTION OF SOMATIC EMBRYOGENESIS IN CALLUS CULTURES OF *ALLIUM* CROP SPECIES AND PLANT REGENERATION

EWA GRZEBELUS, KATARZYNA STELMACH-WITYK, KAMIL SZYMONIK, DARIUSZ KADLUCZKA

Department of Plant Biology and Biotechnology, Faculty of Biotechnology and Horticulture, University of Agriculture in Krakow, Mickiewicza 21, 31-120 Kraków, Poland, e-mail: ewa.grzebelus@urk.edu.pl

Somatic embryogenesis is the preferred method of morphogenesis in plant *in vitro* cultures. Such a developmental pathway is particularly valuable in crop improvement applications based on somatic hybridization or genome editing. The aim of the present study was to develop a reliable protocol for the efficient regeneration of plants from somatic embryos of *Allium* crops.

Eighteen garlic, onion and shallot cultivars/breeding lines were tested for their ability to callus development. Callus was induced from garlic clove fragments or from zygotic embryos isolated from onion and shallot seeds on BDS (Dunstan and Short, 1977) media supplemented with 2,4-D or 2,4-D and BAP. Induction of somatic embryogenesis was examined on callus cross sections. Callus was embedded in Technovit resin and after staining of sample sections with toulidine blue their status was examined under microscope. The regenerative capacity of selected callus lines was tested on B5 (Gamborg et al., 1968) or BDS based media, hormone-free or supplemented with NAA or 2iP, with different amount of sucrose.

Callus development was observed both on clove fragments and zygotic embryos in all examined garlic, onion and shallot cultivars/breeding lines, respectively. Efficiency of callus induction was different and ranged in garlic from 15% to 92%, in onion from 4% to 80% while in shallot from 46% to 92%. Similar number of explants produced callus on both tested culture media, however differences between used media were observed for individual garlic onion and shallot cultivars/breeding lines. Among the different types of callus, a preferred friable callus was observed for all examined accessions. Histological observation of exemplary accession of garlic and onion revealed the presence of globular structures mainly composed of meristematic cells characterized by dense cytoplasm and a round-shaped nucleus. Applied regeneration media allowed for plant development both from garlic, onion and shallot callus.

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THE INFLUENCE OF TEMPERATURE ON THE COURSE OF EMBRYONIC AND LARVAL DEVELOPMENT OF ALLOTRIPLOID *COBITIS* FISH (TELEOSTEI, COBITIDAE) REARED UNDER CONTROLLED CONDITIONS

OLGA JABŁOŃSKA, JULIA TYSZKO, ALICJA BOROŃ, DOROTA JUCHNO

Department of Zoology, Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Oczapowskiego 5, 10-959 Olsztyn, Poland, e-mails: olga.jablonska@uwm.edu.pl, julia.tyszko@student.uwm.edu.pl, alibo@uwm.edu.pl, juchno@uwm.edu.pl

Temperature is one of the most important environmental factors affecting fish development. In recent years, increasing attention has been paid to its impact on aquatic organisms in the context of ongoing climate change. Freshwater habitats throughout Poland are inhabited by *Cobitis* fish, which constitute an excellent model for studies on hybridization, polyploidization, and the influence of temperature on reproductive modes and developmental processes. Within the genus *Cobitis*, both sexual species - *C. taenia* and *C. elongatoides* - as well as polyploid forms, including triploid females and tetraploid males and females, can be distinguished (Juchno et al., 2014). Triploid females reproducing gynogenetically are the most abundant component of mixed populations and can "decide" whether their eggs develop gynogenetically, clonally, or undergo fertilization, resulting in tetraploid offspring. Temperature may influence this process.

The aim of this study was to investigate the effect of temperature on the embryonic and larval development of offspring derived from triploid *Cobitis* females. Females were collected from the Pilica River and males from the Łyna River (12 individuals in total) and hormonally stimulated prior to spawning. Triploid *Cobitis* females were crossed with *C. taenia* males. The obtained embryos and larvae were incubated at: 18°C, 22°C, and 28°C and observed over six consecutive days.

Embryonic and larval development followed the same sequence of stages at all temperatures; however, higher temperatures significantly accelerated the rate. Time from fertilization to hatching was approximately 74 h at 18°C, 63 h at 22°C, and 33 h 51 min at 28°C. Developmental abnormalities occurred at all temperatures, with the highest diversity at 28°C.

These results indicate broad thermal tolerance and high adaptive potential of *Cobitis* fish to changing thermal conditions in freshwater ecosystems.

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Reorganization of chromatin configuration in oat × maize addition lines

Katarzyna Juzoń-Sikora[1], Anna Nowicka[2], Mahmoud Said[2,3], Dominika Idziak-Helmcke[4], Tomasz Warzecha[5], Marzena Warchoł[1], Edyta Skrzypek[1], Ales Pecinka[2]

[1]*The Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków, Poland, e-mail: k.juzon@ifr-pan.edu.pl, m.warchol@ifr-pan.edu.pl, e.skrzypek@ifr-pan.edu.pl*

[2]*Centre of Plant Structural and Functional Genomics, Institute of Experimental Botany of the Czech Academy of Sciences, Olomouc, Czech Republic, e-mail: nowicka@ueb.cas.cz, said@ueb.cas.cz, pecinka@ueb.cas.cz*

[3]*Field Crops Research Institute, Agricultural Research Centre, Giza, Egypt, e-mail: said@ueb.cas.cz*

[4]*Institute of Biology, Biotechnology, and Environmental Protection, University of Silesia in Katowice, Katowice, Poland, e-mail: dominika.helmcke@us.edu.pl*

[5]*Department of Plant Breeding, Physiology, and Seed Science, University of Agriculture in Kraków, Kraków, Poland, e-mail: tomasz.warzecha@urk.edu.pl*

The oat (*Avena sativa* L.) × maize (*Zea mays* L.) addition (OMA) lines constitute a unique tool for studying gene expression and the structure of chromatin as well as a source of agronomically desirable traits in the context of new intergeneric hybrid cultivars. Therefore, for a better understanding of the introgressions of maize chromatin into the oat genome, a comprehensive OMA analysis was conducted.

The presence of maize chromatin was confirmed in three tested lines by PCR-based amplification of the maize retrotransposon *Grande-1*, and verified by genomic *in situ* hybridization. Interestingly, the OMA lines containing cytologically detectable maize chromatin lost the Rabl interphase chromosome configuration typical for oat. Moreover, the maize chromatin was consistently positioned near the nuclear periphery. The maize chromosome additions caused a prolonged DNA replication phase, altering cell cycle dynamics, and led to mitotic abnormalities, as evidenced by the formation of micronuclei and anaphase chromosome bridges. The OMA lines also showed smaller seeds, hampered seedlings' growth, and reduced number of seeds per plant and seed weight.

The results suggest that the complexity of OMA functioning seemed to be influenced by the specific maize chromosome and its interaction with the oat genome, rather than only the number of retained chromosomes. Further studies of OMA lines will be essential to assess their long-term stability and to appreciate their full potential in both basic research and crop improvement.

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PECTINS AND ARABINOGALACTAN PROTEINS AS MARKERS OF DROUGHT-INDUCED CHANGES IN MALE AND FEMALE REPRODUCTIVE TISSUES OF CARROT (*DAUCUS CAROTA* L.)

MAŁGORZATA KAPUSTA[1], MAŁGORZATA CZERNICKA[2], BARBARA JAGOSZ[2], EMILIA WILMOWICZ[3], AGATA KUĆKO[4], JOANNA KOCIĘCKA[5], STANISŁAW ROLBIECKI[6], ROMAN ROLBIECKI[6], JAKUB MIĘTKI[3]

[1]*Bioimaging Laboratory, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland*

[2]*Department of Plant Biology and Biotechnology, University of Agriculture in Krakow, al. Mickiewicza 21, 31-120 Krakow, Poland*

[3]*Department of Plant Physiology and Biotechnology, Nicolaus Copernicus University, Lwowska 1, 87-100 Toruń, Poland*

[4]*Department of Plant Physiology, Institute of Biology, Warsaw University of Life Sciences-SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland*

[5]*Department of Land Improvement, Environmental Development and Spatial Management, Poznan University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland*

[6]*Department of Biogeochemistry, Soil Science, Irrigation and Drainage, Bydgoszcz University of Science and Technology, Bernardynska 6, 85-029 Bydgoszcz, Poland, e-mail: malgorzata.czernicka@urk.edu.pl*

Drought during the generative phase can impair carrot reproductive performance by reducing pollen viability and altering cell wall mechanics, thereby limiting pollination and fertilisation efficiency. Cell wall remodelling is a key component of these processes; however, tissue-specific markers of drought-induced changes in reproductive structures remain poorly characterised. Identifying cell wall components responsive to water deficit may provide candidate markers for the selection of genotypes differing in drought sensitivity. This study examined whether pectins and arabinogalactan proteins (AGPs), which are involved in cell wall hydration status, mechanical properties, and pollen tube growth, can serve as markers of drought-induced changes in male and female reproductive tissues of *Daucus carota* L. Five accessions ('Dolanka', DC295, DC359, DC522 and DC701) were selected based on prior physiological characterisation, with DC295 classified as more drought-sensitive and the remaining accessions as relatively more tolerant (Jagosz et al., 2025). Pollen viability and preliminary immunolocalisation analyses using JIM5, JIM7, JIM8 and JIM13 antibodies were performed in selected somatic and reproductive tissues under control and drought conditions. Pollen viability differed among accessions, being highest in DC359 and DC522, intermediate in DC701, lower in DC295, and lowest in 'Dolanka'. Preliminary mean fluorescence intensity (MFI) measurements indicated drought-related changes in pectin epitopes, particularly those recognised by JIM5. The strongest increase in JIM5 signal under drought conditions, relative to the respective controls, was observed in the drought-sensitive accession DC295, in both somatic and generative tissues. A similar but less pronounced increase was detected in DC359, whereas no comparable drought-induced increase was observed in 'Dolanka' or DC522 in the initial measurement series. Responses of other epitopes (JIM7, JIM8 and JIM13) were weaker and showed genotype- and tissue-dependent patterns, precluding general conclusions at this stage. These results suggest that specific pectin epitopes, particularly JIM5, may have potential as markers of drought-associated cell wall remodelling in carrot reproductive tissues. However, further quantitative and spatial analyses are required to confirm their reliability and functional relevance.

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ASSESSMENT OF ANDROGENIC RESPONSE OF HETEROZYGOUS BREEDING MATERIALS OF RADISH (*RAPHANUS SATIVUS* L.) IN ANTHER CULTURE

WALDEMAR KISZCZAK, MARIA BURIAN

The National Institute of Horticultural Research,
Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland,
e-mail: waldemar.kiszczak@inhort.pl

Radish (*Raphanus sativus* L.) is regarded as a species with low responsiveness to microspore embryogenesis and doubled haploid production, which makes the development of efficient in vitro protocols difficult (Kozar et al., 2021; Chen et al., 2022). The aim of this study was to evaluate the androgenic potential of heterozygous radish breeding materials supplied by breeders using anther culture. Flower buds containing more than 50% of microspores at the late uninucleate and early binucleate stages were selected for culture initiation. After surface sterilization, anthers were isolated and cultured on solid induction media based on Gamborg B5 medium (Gamborg et al., 1968). Two variants were tested: B5-3R supplemented with NAA (0.1 mg L^{-1}) and 2,4-D (0.1 mg L^{-1}), and B5-4R supplemented with 2,4-D (0.3 mg L^{-1}). For genotype POL-1, 0.2 and 0.6 structures per 100 anthers were obtained on B5-3R and B5-4R, respectively, whereas for POL-3 the values were 0.9 and 1.5, indicating a genotype-dependent response, as also reported for radish microspore embryogenesis by other authors (Chun et al., 2011; Chen et al., 2022). Anthers cultured on the more effective medium were then exposed to low (4°C) or high (35°C) temperature for 1-8 days to redirect microspore development from the gametophytic to the sporophytic pathway. Low temperature was generally more effective than heat treatment. The highest response was observed for POL-1 after 4 days at 4°C (10.6 structures per 100 anthers), while for POL-3 the optimum response was obtained after 6 days at 4°C (1.1 structures per 100 anthers). No direct gametic embryogenesis was observed; however, indirect embryogenesis through callus formation occurred.

ACKNOWLEDGEMENTS

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Evaluation of survival and developmental rates of *Drosophila melanogaster* (Drosophilidae) larvae exposed to C60 and C70 fullerenes

Kamil Kosiorowski[1], Karol Małota[2], Magdalena Rost-Roszkowska[2], Maciej Serda[3]

[1]*College of Interdisciplinary Studies at the University of Silesia in Katowice, Bankowa 12, 40-007 Katowice, Poland, e-mail: kamilkosiorowski1999@gmail.com*

[2]*Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, 40-007 Katowice, Poland*

[3]*Institute of Chemistry, University of Silesia in Katowice, Szkolna 9, 40-006 Katowice, Poland*

Recently, fullerene nanomaterials have gained significant scientific recognition for their unique physico-chemical properties. These characteristics allow for a range of applications across diverse fields from medicine and pharmacology to cosmetology, electronics, and material engineering. However, due to its intensive industrial interest and wide usage, it leads to its increasing presence in the environment. Understanding their effects on organisms undergoing complete metamorphosis is crucial, not only from an ecotoxicological point of view but also as a potential new insect control agent.

Drosophila melanogaster remains an invaluable biological and ecological model for such research. As one of the most widely studied and documented organisms in biology, it offers a rapid and precise *in vivo* platform for assessing toxicological, developmental impacts of environmental stressors.

The aim of the study was to determine the survival rate of *D. melanogaster* larvae in the presence of C_{60} and C_{70} fullerenes in the environment.

Three groups were established: experimental C_{60}, $C_{70,}$ each separately at a concentration of 1mg/mL, and a control group. For each group, 1mL of Nutri-Fly® Bloomington Formulation food was applied to Petri dishes containing a supplemented solution of fullerenes in ddH_2O, or to Petri dishes containing ddH_2O without fullerenes. Eggs were collected from a laboratory culture of *D. melanogaster*, originally obtained from the Vienna Drosophila Resource Center. For each of the groups, 100 eggs were placed on the surface of the treated medium and incubated at the optimal temperature of 24°C.

To determine whether the tested substances influenced larval metamorphosis into adult flies, firstly, the number of eggs placed into each Petri dish was recorded. Larval development was monitored daily until adult flies emerged. Based on the observations, the metamorphosis success rates were calculated for the control group, which served as the reference, and for all experimental treatments.

The larval survival rate has revealed significant differences between the groups. In the control group, larval metamorphosis success rate was the highest, while in the C_{70} group, it was the lowest.

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The effect of Wi-Fi radiation on the ultrastructural organization of the glandular stomach in chicken embryos

Magdalena Kowalska[1], Magdalena Rost-Roszkowska[1], Sandra Andrašková[2], Viera Almášiová[2], Patrícia Hudáková[2], Ján Molnár[3], Paweł Kaczmarek[1], Katarína Holovská [2]

[1]*Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, Bankowa 9, Katowice, Poland, e-mail: magdalena.rost-roszkowska@us.edu.pl*

[2]*Department of Morphological Disciplines, University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, Košice, Slovakia*

[3]*Department of Theoretical and Industrial Electrical Engineering, Faculty of Electrical Engineering and Informatics, Technical University, Letná 9, Košice, Slovakia*

Wireless smart technologies are widely adopted. Their radiation is generally considered low; however, it may still affect young, developing organisms, which are especially sensitive to a range of external influences, including non-ionizing radiation (NIR).

The chicken embryo is a suitable model for studying the effects of Wi-Fi radiation on organ development due to its rapid growth, accessibility, and the straightforward application of non-ionising radiation. The chicken stomach consists of two distinct parts: the glandular section (proventriculus), involved in enzymatic digestion, and the muscular section (gizzard), which grinds food.

This study aimed to investigate how Wi-Fi radiation at 2.4 GHz, with a power density of 200–500 $\mu W/m^2$, affects the glandular stomach of 14-day-old chicken embryos.

The experiment used fertilised chicken eggs from a certified breeding facility in Párovské Háje, Nitra, the Slovak Republic. The eggs were divided into two groups: a control group (not exposed to Wi-Fi radiation), and an experimental group (exposed to Wi-Fi radiation). For transmission electron microscopy (TEM) analysis, the organs were isolated, fixed, and prepared according to standard protocols.

At the ultrastructural level, changes in the Wi-Fi–exposed embryos were detected in the connective tissue of the propria-submucosa surrounding the epithelium. The cells in this tissue were more loosely arranged, with increased intercellular spaces and a higher fiber abundance than in the control group. Moreover, a large number of apoptotic cells in the connective tissue was observed in embryos exposed to Wi-Fi radiation.

The findings of this study may help deepen our understanding of how Wi-Fi radiation interacts with pre-hatching development.

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IN DEVELOPING OOCYTES OF BASAL DERMAPTERANS LYSOSOME-LIKE ORGANELLES ARE DISTRIBUTED UNIFORMLY AND DO NOT ASSEMBLE AT THE POSTERIOR POLE, AS IT IS IN DERIVED SPECIES

STANISLAW MIODONSKI[1], PAULA IRLES[2], ANDRES F. SARRAZIN[3], WACLAW TWORZYDLO[1], MAGDA SOCHACZEWSKA[1,4], SZCZEPAN BILINSKI[1]

[1]*Department of Developmental Biology and Invertebrate Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland*

[2]*Institute of Agri-food, Animal and Environmental Sciences, Universidad de O'Higgins, Rancagua, Chile*

[3]*Institute of Chemistry, Pontifical Catholic University of Valparaiso, Valparaiso, Chile*

[4]*Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland, e-mail: szczepan.bilinski@uj.edu.pl*

Ovaries of basal dermapterans, *Gonolabina spectabilis* (Pygidicranidae) and *Euborelia annuilipes* (Anisolabididae) are composed of five elongated functional units, *i.e.* the ovarioles. Each ovariole consists of four morphologically recognizable regions: an apical terminal filament, germarium, vitellarium, and an ovariole pedicel. The vitellarium houses several (up to 10) ovarian follicles arranged linearly, in a way that the gradually older follicles are located more basally. As in other dermapterans, the ovarian follicles are relatively simple and comprise two germ-line cells only: an oocyte and a single nurse cell. These cells are connected *via* cytoplasmic canal (intercellular bridge). The nurse cells are synthetically highly active and possess large nuclei with ramified nuclear envelope. During successive stages of oogenesis, the oocytes increase in size accumulating various organelles (mitochondria, lysosome-like bodies, elements of endoplasmic reticulum) and reserve materials (yolk spheres and lipid droplets) in the ooplasm. Simultaneously, the oocyte nucleus (germinal vesicle) relocates from the neighbourhood of the intercellular bridge to the future dorsal side of the embryo.

In the preceding paper, we have shown that in derived dermapterans (*Forficula auricularia*, *Apterygida media*) all lysosome-like bodies present in the ooplasm are transferred toward the posterior pole and form there a distinct compartment, termed posterior pole lysosomal compartment. Interestingly, in the studied basal dermapterans all oocyte organelles, including lysosome-like bodies, remain evenly dispersed throughout the ooplasm till the final stages of oogenesis. Based on these observations, we conclude that the oocytes of basal earwigs lack the posterior pole lysosomal compartment, and that this organelle assemblage is characteristic of derived dermapterans (Eudermaptera) only.

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MICROSATELLITE POLYMORPHISM IN *TARAXACUM* SEEDS

PATRYK MIZIA[1], JOLANTA MARCINIUK[2], PAWEŁ MARCINIUK[2], ALEKSANDRA GRABOWSKA-JOACHIMIAK[3], ANDRZEJ JOACHIMIAK[1]

[1]*Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University, Gronostajowa St. 9, 30-387 Kraków, Poland, e-mail: andrzejot@gmail.com*

[2]*Institute of Biological Sciences, Faculty of Natural Sciences, University of Siedlce, Bolesława Prusa St. 14, 08-110 Siedlce, Poland, e-mail: jolanta.marciniuk@uws.edu.pl, pawel.marciniuk@uws.edu.pl*

[3]*Department of Plant Breeding, Physiology and Seed Science, University of Agriculture in Kraków, Łobzowska St. 24, 31-140 Kraków, Poland, e-mail: aleksandra.grabowska-joachimiak@urk.edu.pl*

The genus *Taraxacum* is a model for research on apomixis. It includes diploid, sexually reproducing species, as well as polyploid (mostly tri- and tetraploids) apomicts. Their apomixis is often described as obligate, although since the 1970s it has been known that they can form hybrids with diploid species, thus participating in sexual reproduction. This is possible because many of them are capable of producing fertile pollen which, when deposited on the stigma of diploid flowers, can contribute to the formation of hybrid forms with varying ploidy levels. This is believed to be the main source of variation in the genus *Taraxacum*, leading to the formation of both secondary, sexually reproducing diploids and popliploids, which can give rise to new apomictic species.

While the role of apomicts as paternal plants in the production of hybrid offspring by diploids is unquestionable, the ability of apomictic mothers to produce offspring on the sexual way remains controversial. Despite sporadic embryological observations indicating their residual sexuality, this is generally considered unlikely under natural conditions due to the mechanism of premature embryo development in closed (and therefore inaccessible to pollen) flowers. To determine whether this mechanism actually prevents the production of hybrid offspring, microsatellite profiles of seeds produced in open-pollinated flower heads of six plants belonging to two apomictic forms of *Taraxacum* from the section *Palustria* were examined. Seeds from bagged capitula were used as controls. In five plants, all seeds produced by both isolated and non-isolated inflorescences were found to have originated apomictically. Their microsatellite profiles were identical to those of the parent plants. However, in one plant, the microsatellite profiles of some seeds collected from the non-bagged capitula contained additional microsatellites and the full complement of maternal microsatellites, which suggested their hybridity. Flow cytometric studies confirmed this possibility, demonstrating that some seeds from this inflorescence contained embryos with a higher ploidy level than the parent plant.

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Analysis of the cell cycle and proliferation of midgut stem cells in freshwater and soil crustaceans exposed to selected xenobiotics

Anna Ostróżka[1], Magdalena Rost-Roszkowska[1], Grażyna Wilczek[1], Łukasz Chajec[1], Agata Aleksa[1], Hanna Gorol[1], Adrianna Dołęgowska[1], Martyna Lalik[2], Dominika Dąbrowska[2]

[1]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Bankowa 9 Katowice, 40-007 Poland, e-mail: anna.ostrozka@us.edu.pl*

[2]*University of Silesia in Katowice, Institute of Earth Sciences, Będzińska 60, 41-200 Sosnowiec, Poland*

The midgut epithelium of Crustacea is composed of different types of cells which differentiate from the midgut stem cells (embryonic cells, regenerative cells). Thanks to their ability to divide, midgut stem cells can increase in number and are responsible for epithelial self-renewal. However, numerous stressors which originate from the environment can disrupt the digestive epithelium, causing inflammation or even death. The aim of our study was to analyze changes in midgut stem cells' proliferation and the cell cycle caused (or not) by different stressors. Crustaceans inhabiting diverse environments were selected for this study, along with various xenobiotics whose environmental concentrations are increasing due to anthropogenic activity. The midgut was chosen as a model organ in toxicological research because it constitutes, alongside the body surface, a key barrier between the organism and the external environment. Disturbances in its structure and function may affect the homeostasis of the entire organism.

The aim of this study was to evaluate the mitotic activity of midgut stem cells in selected freshwater crustaceans, *Neocaridina davidi* (Malacostraca), and terrestrial species, *Porcellio scaber* and *Oniscus asellus* (Isopoda), under exposure to selected xenobiotics. Additionally, the proliferative potential of these cells was assessed. Shrimps (*Neocaridina davidi*) were exposed to an aqueous solution of nickel (8 mg $NiCl_2$/L) for one and two weeks, as well as to bisphenol solutions at concentrations of 1, 5, and 10 mg/L for 24, 48, and 72 hours. Terrestrial crustaceans (*Porcellio scaber* and *Oniscus asellus*) were exposed to industrial and municipal leachates at concentrations of 20% and 50% for one and three months. Subsequently, the midgut was isolated for analysis. To achieve the study objectives, flow cytometry (Muse system) was applied, along with Cell Cycle and Ki-67 assays to evaluate cell proliferation and mitotic activity.

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Sexual reproduction of CRISPR/Cas9-mediated methyltransferase mutants of Tartary buckwheat (*Fagopyrum tataricum* L.)

Aneta Słomka[1], Magdalena Zaranek[1,2], Artur Piński[2], Klaudia Sychta[1], Agnieszka Płażek[3], Przemysław Kopeć[4], Ewa Grzebelus[5], Alexander Betekhtin[2]

[1]*Institute of Botany, Jagiellonian University, Gronostajowa 9, 30-387 Kraków, Poland, e-mail: aneta.slomka@uj.edu.pl, magdalena.zaranek@uj.edu.pl, klaudia.sychta@uj.edu.pl*

[2]*Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Jagiellońska 28, 40-032, Katowice, Poland, e-mail: artur.pinski@us.edu.pl, alexander.betekhtin@us.edu.pl*

[3]*Department of Physiology, Plant Breeding and Seed Science, University of Agriculture in Krakow, Adama Mickiewicza 21, 31-120 Kraków, Poland, e-mail: rrplazek@cyf-kr.edu.pl*

[4]*Polish Academy of Sciences, Institute of Plant Physiology, Niezapominajek 21, 30-239 Kraków, Poland, e-mail: p.kopec@ifr-pan.edu.pl*

[5]*Department of Plant Biology and Biotechnology, University of Agriculture in Krakow, Adama Mickiewicza 21, 31-120 Kraków, Poland, e-mail: ewa.grzebelus@urk.edu.pl*

Tartary buckwheat (*Fagopyrum tataricum* L.) is a valuable medicinal crop rich in flavonoids. The CRISPR/Cas9 system has emerged as an efficient tool for precise genome editing in this species, facilitating both gene function research and crop quality improvement. To explore its genetic regulation, CRISPR/Cas9-generated mutants of *methyltransferase 1* (*met1*) and *methyltransferase 2* (*met2*) were cultivated under long-day (LD) and short-day (SD) conditions. We investigated how photoperiod-induced energy limitations affect the reproductive success of these epigenetic mutants.

Results revealed a critical post-fertilization bottleneck rather than gametophytic sterility. Although *met1* showed slightly reduced pollen viability (~83%) and *met2* remained wild-type-like, both lines possessed normal embryo sacs. Thus, the drastic yield reduction occurs during seed development. In starch-rich buckwheat seeds, *MET1* and *MET2* appear essential for the genomic imprinting required for proper endosperm formation. A strong positive correlation between dry shoot mass and yield indicates that these mutants are primarily resource-limited. While the wild-type remains stable across photoperiods, both *met* mutants exhibit extreme sensitivity to the energy deficit of SD.

We hypothesize that limited carbon resources in SD exacerbate the developmental instability of *met1* and *met2* seeds, leading to widespread abortion and a failure to complete the maturation program, even when initiated by viable gametes.

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MORPHOLOGY AND REPRODUCTIVE BIOLOGY OF *TRICHOSANTHES KIRILOWII* MAXIM. INTRODUCED TO CENTRAL EUROPE

DOROTA TCHÓRZEWSKA, KRYSTYNA WINIARCZYK

Department of Cell Biology, Institute of Biological Sciences, Maria Curie-Skłodowska University, Akademicka 19, 20-033 Lublin, Poland, e-mail: dorota.tchorzewska@mail.umcs.pl, krystyna.winiarczyk@mail.umcs.pl

Trichosanthes kirilowii (*Cucurbitaceae*) represents an interesting model for studies on reproductive biology; however, detailed data on the ontogeny and phenology of this species under the climatic conditions of Central Europe remain lacking. The aim of the present study was to analyze sexual polymorphism and the inflorescence architecture of *T. kirilowii*. The flowers of the investigated species are characterized by white perianth petals terminated by filamentous appendages (fringes), which play an important role in attracting pollinators during nocturnal anthesis. Previous literature data on the sexual system of this plant indicate that it is a dioecious species with unisexual flowers (Hu et al., 2020). However, our observations demonstrated that the investigated population exhibits sexual polymorphism: in addition to male individuals, individuals bearing bisexual flowers were recorded, indicating androdioecy; these flowers possessed a single pistil and three reduced stamens fused to the perianth. Furthermore, in contrast to literature reports describing solitary positioning of female flowers (DeWilde and Duyfjes, 2008), some individuals exhibited acropetal development within a lax raceme. In addition, inhibition of the development of the youngest floral buds along the axis was observed following successful pollination of earlier-developed flowers, which markedly limits the final number of fruits. The results obtained indicate considerable developmental plasticity in *T. kirilowii* and suggest that the sex determination system in this species is more complex than previously assumed. Further studies on the ontogeny of bisexual flowers may provide valuable insights into the mechanisms underlying the evolution of androdioecy in the *Cucurbitaceae* family. Moreover, the observed differences in inflorescence architecture and reproductive system constitute an important contribution to understanding the biology of a species introduced into novel climatic conditions. It is worth emphasizing that elucidation of the mechanisms limiting fruit set, such as inhibition of bud development, is essential for optimizing the cultivation of this valuable medicinal plant in Central Europe.

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THE ROLE OF DAIDZIN IN SOMATIC EMBRYOGENESIS INDUCTION IN *ARABIDOPSIS THALIANA*

VIKTORIIA VEREZUNSKA, ANNA M. WÓJCIK

University of Silesia in Katowice, Faculty of Natural Science, Institute of Biology, Biotechnology and Environmental Protection, Jagiellońska 28, 40-032 Katowice, Poland, e-mail: vverezunska@us.edu.pl, anna.wojcik@us.edu.pl

Somatic embryogenesis (SE) is a crucial tool in plant biotechnology and involves the reprogramming of somatic cells into embryos, reflecting plant developmental plasticity. Auxin is the primary inducer of this process, and its spatial distribution determines the initiation of the embryogenic pathway. Transcription factors play a major role in SE induction; among them, the *WOX5* (*WUSCHEL-RELATED HOMEOBOX 5*) is essential for maintaining the stem cell niche and coordinating cellular reprogramming. We previously identified *WOX5* as a key regulator in auxin-dependent *in vitro* regeneration, showing that its overexpression triggers SE even on auxin-free media (Wójcik et al., 2025).

Recent studies indicate that daidzin, an isoflavone, modulates auxin levels and transport by influencing *WOX5* activity in *Arabidopsis* roots (Yue et al., 2025). In this study, we tested whether the daidzin-WOX5 mechanism induces SE and identified the genes involved in this response. Cotyledon-stage embryos from the Col-0 ecotype and the *wox5-1* mutant were cultured on Gamborg E5 and E0 media supplemented with varying concentrations of daidzin. SE efficiency was evaluated after 21 days. To analyze the molecular mechanism, WOX5 expression was visualized using a *35S:WOX5-GFP* reporter line, and expression profiles of SE marker genes were measured via RT-qPCR.

Our results suggest daidzin modulates auxin signaling, transport, and biosynthesis, affecting the spatial organization and activity of key developmental regulators, particularly *WOX5*, and *PLETHORA* genes, thereby influencing the embryogenic potential of somatic cells in *Arabidopsis thaliana*.

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BERGRREN DJ. 1981. *Atlas of Seeds*, part 3. Swedish Museum of Natural History, Stockholm.

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