Searching at the base of the Pancrustacea tree. Prof. Kubrakiewicz's interest in oogenesis of crustaceans

Mariusz K. Jaglarz

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

The late Prof. Janusz Kubrakiewicz's fascination with crustaceans goes back to the early 1990s. He then turned his interest to other arthropod groups, but resumed the study of the ovary structure and oogenesis of crustaceans in the last years of his scientific activity. I feel fortunate and privileged to have had the opportunity to collaborate with Prof. Kubrakiewicz in this area of research. Inspired by the most recent concepts in arthropod phylogeny, the Pancrustacea/ Tetraconata hypothesis, our research group analyzed the female gonad organization, at both the histological and ultrastructural level, in two crustacean groups: Branchiopoda and Remipedia. The examination of branchiopods belonging to Spinicaudata and Laevicaudata groups demonstrated that the ovaries of these crustaceans comprise numerous ovarian follicles, which protrude into the body cavity, contain linear non-branched germline cysts and are surrounded by a characteristically organized follicular epithelium (Jaglarz et al., 2014b). Each germline cyst consists of four cells, which during oogenesis differentiate into a single oocyte and three nurse cells, connected with the oocyte by means of intercellular bridges. The combined results indicated that the ovaries of these branchiopods are of the meroistic type and share, rather unexpectedly, certain morphological similarities with the basal hexapods. Owing to Prof. Kubrakiewicz's scientific contacts, our group had the opportunity to examine the female reproductive system of Godzilliognomus frondosus, a representative of Remipedia – rare yet important phylogenetically crustaceans. Our comprehensive analysis revealed that significant similarities, in the ovary organization and ultrastructural aspects of oogenesis, are shared by two crustacean groups: Remipedia and Cephalocarida, as well as the basal hexapod lineages: Protura, Collembola and Diplura: Campodeina (Kubrakiewicz et al., 2012; Jaglarz et al., 2014a). The obtained results provided much needed morphological support for a postulated close relationship between these arthropod groups based on the analysis of molecular characters.

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Prof. Kubrakiewicz's interest in neuropterid insects. Comparative studies of the germ cells formation, ovariole organization and course of oogenesis

Izabela Jędrzejowska

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

Prof. Kubrakiewicz's long-standing fascination with oogenesis of Neuropterida dates backs to the early 1990s. His scientific interest was shared with a group of co-workers from Jagiellonian University. I was honored to participate in some contributions.

The superorder Neuropterida comprises three orders: Neuroptera, Raphidioptera and Megaloptera. Comparative studies of the ovariole organization revealed that in neuropterids three types of the ovarioles structure exist. In Neuroptera the ovarioles are polytrophic and germ cell clusters are linear, hardly ramified. Variable and unfixed number of cystocytes results from asynchronous divisions. Among neuropterans Coniopterygidae exhibit a few peculiar characters. Ovaries of Raphidioptera and Megaloptera: Sialidae are of a distinctive telotrophic type, whereas the ovarioles of Megaloptera: Corydalidae are neopanoistic. Data from comparative analysis of the ovariole organization was used in phylogenetic considerations and a hypothesis of paraphyletic origin of Megaloptera was supported (Kubrakiewicz et al., 1998).

Our in depth investigations concerned the course of the ovariole development and formation and structure of nutritive cords in telotrophic ovaries of Raphidioptera (Jędrzejowska and Kubrakiewicz, 2002, 2004).

Comparative analyses of development and structure of the micropyle in neuropterid insects showed that the eggshells of studied neuropterids display great morphological similarities although some discrepancies were also found (Kubrakiewicz et al., 2005).

Our the last contribution was devoted to a modified pattern of the follicular cells diversification and the egg shell structure in *Osmylus fulvicephalus*. In *Osmylus* the eggshell displays dorso-ventral polarity which was found to be a unique feature among neuropteran insects (Garbiec et al., 2015).

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Embryological investigation of apomictic taxa conducted at the Department of Plant Cytology and Embryology of the Jagiellonian University – a continuation of research initiated by Professor Romana Czapik

Maria Kościńska-Pająk, Krystyna Musiał

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

Scientific activity of Professor Romana Czapik, who was an unquestioned authority in the field of apomixis, was focused especially on reproductive processes in apomictic seed plants. Professor Czapik invariably emphasized that the investigation of embryological processes is crucial for the understanding of apomixis (Czapik and Kościńska-Pająk, 2000). The importance of such studies is also stressed in the present research on molecular mechanisms and genetic background of apomixis. After Professor Czapik's death, the embryological studies of apomictic taxa have been continued by her students.

Current research is conducted especially on selected species representing the agamic complexes of *Taraxacum* and *Chondrilla*. Our comparative study of the amhimictic and apomictic taxa seems to be particularly valuable. The present study concerns mainly: (i) the configuration of tubuline cytoskeleton during microsporogenesis as well as in the cells of the egg apparatus (Kościńska-Pająk, 2006; Kościńska-Pająk and Bednara, 2006; Musiał and Kościńska-Pająk, 2013), (ii) callose deposition during megasporogenesis (Musiał et al. 2015), and (iii) developmental processes in anthers of male sterile dandelions (Janas et al. 2016). It is worth mentioning that the immunocytochemical analysis of cytoskeleton configuration was used for the first time in reference to apomictic plants. In our investigation of apomictic processes we used fluorescent microscopy, Nomarski DIC optic, TEM, clearing tissue technique, and different cytochemical methods.

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Responses of plants to polluted with heavy metals environment: from embryology to genetic differentiation. The continuation of the research initiated by Professor Romana Czapik

Monika Kwiatkowska*, Aneta Słomka, Elżbieta Kuta

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

Imbalanced level of metals in soil, resulting from anthropogenic impact on the environment may affect biodiversity and ecosystems via their negative influence on all organisms (Hautier et al., 2015).

Studies on embryological processes in ovules and anthers in plants colonizing post-industrial areas were initiated almost twenty years ago by Professor Romana Czapik (Czapik, 2002) and are continued till today in the Department of Plant Cytology and Embryology of the Jagiellonian University (reviewed in Izmaiłow at al., 2015).

The new approaches to the impact of polluted environment on plants included further analysis of embryological processes with the use of different techniques (*Armeria, Biscutella, Cardaminopsis, Capsella, Cirsium, Chondrilla, Conyza, Corynephorus, Echium, Festuca, Lotus, Medicago, Vicia, Viola*), physiological reaction on stress, alteration in genome, origin of metal-tolerant genotypes (Viola). It was documented that reduced plant fertility by non-regular embryological processes (in male and female lines) occurred with the highest frequency in species at early stage of the colonization ('new comers') contrary to facultative and obligatory metallophytes with almost normal sexual reproduction. Comparative embryological observations lead to the conclusion that these characters could be useful, complementary to characters at sporophytic level, to examine plant resistance/tolerance to environmental stress as a cost of tolerance to harsh conditions. In terms of our studies, the term ecological plant embryology, proposed by Professor Czapik should be widely accepted and commonly used.

Embryological characters combined with macro- and micromorphological traits, antioxidant enzyme activities and genetic diversity were used to find microevolutionary processes of facultative and obligatory metallophytes from the genus *Viola* (Violaceae). Metalliferous populations of *V. tricolor*, considered as a model species to study tolerance to high Zn/Pb/Cd concentrations in the soil, represent similar mode of antioxidative response as their counterparts at non-metalliferous sites indicating that these genotypes are well adapted to polluted environment. The studies comprised also the origin of obligatory metallophytes of calamine (*V. lutea* subsp. *westfalica* and *V. lutea* subsp. *calaminaria*) and serpentine soils (several Albanian pansies) from the ancestral species or via hybridization from non-tolerant closely related species (Słomka and Kuta, 2015). Such biosystematic approach could add important knowledge to understand plant adaptation and tolerance to polluted with heavy metal areas.

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Diptera follicular cells differentiation in a comparative lens. Professor Kubrakiewicz's interest in flies oogenesis

Marta Mazurkiewicz-Kania

Department of Animal Developmental Biology, Institute of Experimental Biology, Wrocław University, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: marta.mazurkiewicz-kania@uwr.edu.pl

Molecular genetic investigations carried on *Drosophila* brought spectacular results and considerably contributed to our understanding of some basic cellular events and their developmental significance. It is now reasonable to ask whether described mechanisms of basic developmental processes are shared by other insects. It can only be resolved through comparative investigations. Such investigations have recently been undertaken by Professor Kubrakiewicz's team in cooperation with Professor Biliński group from Jagiellonian University. I had the honor to participate in this long-term cooperation. Comparative analyses of the follicular cell morphogenesis in the egg chambers of several dipteran species representing main phylogenetic lineages brought interesting results. These analyses have shown that although the mechanisms of germ- and somatic line cell differentiation recognized in *Drosophila* ovaries are essentially shared by all dipterans, the representatives of various phylogenetic lineages within the Diptera may exhibit significant deviations from the most familiar "basic pattern of cell differentiation" of the *Drosophila*-type (Kubrakiewicz et.al., 2003). These deviations are most often exemplified by different cells' activity and/or their developmental potential but also by diverse timing of developmental events. Significant discrepancies in the mode of differentiation and diversification of follicular cells have been found between the more ancestral Nematocera and derived Brachycera. The major difference concerns the "migratory activity" (Kubrakiewicz et al., 2003, Mazurkiewicz and Kubrakiewicz, 2005, Mazurkiewicz-Kania et al., 2012). In Nematocera, none of the follicular cell subgroups displays any ability to migrate, and thus does not alter their position within egg chambers throughout oogenesis. Interestingly, in all dipteran species studied so far the emergence of the polar cells at the egg chamber extremities was evidenced although their ultimate number and developmental potential was found to vary considerably. Based on these comparative morphological analyses some important conclusions concerning the evolution of the morphogenetic processes have been drawn. For instance, it became clear that dipterans must have evolved a special system of follicular cell patterning that involves polar cells, while ability of some follicular cells for invasive migrations represents an evolutionary novelty that must have arisen in the ancestors of the advanced brachycerans only.

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In vitro culture method as a fascinating tool for understanding of plant development: the contribution of Professor Lesław Przywara

Marzena Popielarska-Konieczna

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

Unique features of plant in vitro methods have been seductive for scientists from the beginning of the idea of plant cell, tissue and organ culture. Professor Lesław Przywara was one of the researchers in Poland who took note of in vitro culture great importance and capability. For this reason he was the co-creator of the plant in vitro laboratory in Institute of Botany at Jagiellonian University in Cracow. Professors broad interests concerned many aspects of plant development, but he focused especially on the experimental embryology. Among the most favorite problems for him were: androgenesis, gynogenesis, in vitro cross pollination, somatic embryogenesis, isolated endosperm and zygotic embryo cultures. During the long stay visit in Department of Scientific and Industrial Research in New Zealand he got to know better the plant in vitro techniques and has been involved in researches on *Actinidia*, *Trifolium* and *Triticum*. He continued interests in these crop plants after home-coming to Cracow.

Professor was the co-worker of experiments concerning androgenesis processes (haploid plant regeneration from microspore) in bread wheat (*Triticum aestivum*). After years this model was contributed to recognizing the temporal-spatial localization of pectins and arabinogalactans in the cell wall. The endosperm tissue (extremely specialized storage tissue in seeds, with higher ploidy level than embryo) was the other scientific object of Professor experiments. The main aim of his investigation was the induction of cell proliferation and plant regeneration from isolated endosperm in kiwifruit (*Actinidia deliciosa*) and bread wheat (*T. aestivum*). The first results were hopeful, thus we continued researches and succeeded with plant regeneration in kiwifruit. Moreover the proliferated endosperm-derived callus tissue turned out useful for studies on callus domains and adventitious shoot buds development. The studies with immunolabelling, transmission and scanning electron microscopies revealed specific features of the cell wall during the morphogenic processes. The experiment with endosperm in cereals was not resulted plant regeneration. However we showed, that isolated immature endosperm continue the development under in vitro conditions. Our results suggest that manipulation of deposition pathways in isolated cultured endosperm of cereals is possible and can serve as tool to understand mechanisms related to endosperm development. The first results of RNAseq analyses revealed a different composition of gene transcipts and higher amount of transcriptome related to some type of prolamine compared to caryopsis at the same age.

Professor Lesław Przywara gave us an impulse to interest the plant in vitro culture. Some of his ideas are still proceeding and the investigations are actually conduct using more advanced techniques at biochemical and molecular levels.

Heteropteran ovaries: the organization of trophic chambers and differentiation of the follicular epithelium – the contribution of Professor Antoni Ogorzałek

Bożena Simiczyjew

Department of Animal Developmental Biology, Institute of Experimental Biology, Wrocław University, Sienkiewicza 21, 50-335 Wrocław, Poland, e-mail: bozena.simiczyjew@uwr.edu.pl

The ovary organization and oogenesis in Heteroptera were the leading topic of the scientific research conducted by Professor Ogorzałek.

The heteropteran female gonads are composed of telotrophic-meroistic ovarioles differentiated into an apically arranged tropharium and a basally located vitellarium. The tropharium (trophic chamber) houses trophocytes (nurse cells) that are connected with oocytes by elongated tubes called nutritive cords. The vitellarium comprises developing oocytes surrounded by a follicular epithelium (ovarian follicles).

Comparative studies on the trophic chambers conducted in collaboration with Professor Ogorzałek show that the organization of a tropharium is variable and differs in primitive versus advanced heteropteran families. In representatives of the primitive families the trophic chambers are built of mononucleate nurse cells while in the advanced heteropterans the tropharia are composed of cytoplasmic lobes that contain several trophocyte nuclei (Simiczyjew et al., 1998).

In the main, the scientific endeavours of Professor Ogorzałek concerned the processes of the differentiation and diversification of the follicular epithelium. A significant and the most valuable part of His research, was devoted to the interactions that take place between the oocyte nucleus and the somatic follicular cells, the outcome suggesting that factors emitted from the oocyte nucleus play an important role in differentiation of follicular cells (Ogorzałek, 1987). Investigations carried out on several heteropteran species reveal that during the course of oogenesis initially uniform group of follicular cells diversify into different number of subpopulations, each of them subsequently forming different parts of the egg capsules (Ogorzałek, 2007).

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Memories of Professor Alicja Górska-Brylass

Dariusz Jan Smoliński

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

Professor Alicja Górska-Brylass created a school of cellular biology based on the use of different embryological models. Professor biography and her scientific activities can be read in the memories written by prof. Elżbieta Bednarska-Kozakiewicz (Bednarska-Kozakiewicz, 2015). During the lecture will be presented research initiated and conducted by the professor and continued by her successors in areas such as: molecular plant embryology, RNA biology, cell imaging and plant cell biology. Will be shown the advantages of embryological models in the research on the molecular and cellular level.

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