Journal of Insect Physiology 145 (2023) 104475

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

# František Sehnal: A project that worked out

Marek Jindra

*Biology Center of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice 370 05, Czech Republic*

ARTICLE INFO

*Keywords:*
František Sehnal
*Galleria mellonella*
Insect endocrinology
Juvenile hormone
Metamorphosis
Silk

ABSTRACT

František Sehnal was a prominent and inspiring figure in many areas of insect science, most notably endocrinology, developmental biology, silk research, and recently insect interactions with genetically modified crops. In this article, I will briefly overview Sehnal's research and other academic and educational activities. I would also like to share my personal experience with František Sehnal as a mentor who drafted, in 1990, a plan for my doctoral thesis: to identify a receptor for juvenile hormone. The project ended up taking more than two decades to complete. While František has passed away, his legacy stays.

## 1. Who was František Sehnal to me

To his acquaintances around the world, František Sehnal was known as Franta. "*How is Franta doing?*" and "*Say hello to Franta*" was to be heard at every insect conference. Until November 2021, when he sadly passed away. František Sehnal was my mentor. He supervised my undergraduate and doctoral studies, which concluded in 1993. It would be difficult for me to comment on his work and contribution to science without describing my personal experience. While necessarily subjective, I hope that my following account on František Sehnal's legacy may be authentic and not excessively boring.

"*Someone who experiments with insects and does not belong to the communist party*", was my preferred profile of a supervisor when I sought a project as a sophomore at the Charles University in Prague. On this specific request, our great biochemistry teacher, Jiří Čerkasov, got on the phone and called František Sehnal. Luckily, Sehnal was willing to supervise students and was hiring for his prospective laboratory, then still under construction in České Budejovice.

I first met František Sehnal in the Orwellian 1984. In his tiny lab, hidden inside a Prague apartment house, he asked: "*Do you read English?*" I thought I did. Proofs of his massive chapter Growth and Life Cycles (Sehnal, 1985) for *Comprehensive Insect Physiology, Biochemistry & Pharmacology* (edited by G.A. Kerkut and L.I. Gilbert) landed in my hands. Although we never discussed the chapter, I was overwhelmed by the breadth of his grasp of insect evolution and development and impressed by the eloquence of his written English. How he could breach the political isolation of our country and publish with world leaders was a puzzle I figured out later. "*Let's begin with practice*", was Sehnal's second line I recall from day one of our encounter. The next thing I saw was me digging in jars full of *Galleria mellonella* larvae over the endless task of separating them from their silk tubes and other inseparable mess they have produced since the same practice a day earlier. I had literally opened my "can of worms".

František Sehnal was not your average mentor. I do not ever recall him "teaching" me anything. One could learn by watching him work. I saw him edit a manuscript, write a letter, prepare a talk. And that was it, my lessons for years to come. Sehnal's supervising meant that he would type (or very neatly handwrite) an outline comprising a whole project in a few precisely formulated items, hand it to me, and mind his own business. He would check on the progress weeks or months later. Meanwhile, I barely got to see him, so troubleshooting problems together was a rare option. The first time he saw my master's and doctoral theses was at the defense.

Looking in retrospect, I have no idea how things could have worked out, but they did somehow, as we turned the master's diploma thesis into my first papers, in *JIP* and *IBMB*, respectively (Jindra and Sehnal, 1989, 1990). Sehnal was brave enough to let me write those papers - another big credit to his mentorship. To me, František Sehnal was a fine mentor precisely because he left the learning up to the student.

## 2. The man of perseverance and vision

The answer to how Sehnal, trapped in the communist Czechoslovakia, could publish with the world leaders is twofold. First, it was his deep knowledge of the subject and genuine passion for science. Second, it was his communication skill. Sehnal had a remarkable ability to maintain friendly relationships, established during a short window of opportunity in the mid-1960′s when the country experienced an episode of relative

*E-mail address:* jindra@entu.cas.cz.

https://doi.org/10.1016/j.jinsphys.2023.104475
Received 8 November 2022; Accepted 4 January 2023
Available online 6 January 2023

freedom. In 1966, Sehnal commenced his postdoc stay at Case Western Reserve University (Cleveland, OH) where he met Howard Schneiderman and other key figures in insect development and endocrinology of that time. The window closed when the Russian tanks invaded Czechoslovakia in 1968 (how timely to remind us *today* who was the enemy then, and now again!). Sehnal's refusal to officially agree with the occupation was not without consequences for his career, namely that he was not allowed to take a leader post corresponding to his scientific capacities. But while suppressed, Sehnal managed to keep his international contacts alive.

The Russian occupants stayed in the country for more than two decades, and so did Franta. Instead of joining luckier colleagues in the free world, he chose to fight his uphill battle under the adverse regime. That was fortunate for several students including myself. Sehnal managed to create a protected niche where motivated people could work. Some of them have contributed to this issue.

In 1985, the government inaugurated academic institutes in the provincial town České Budějovice (aka Budweis in German and for beer connoisseurs). These included the Institute of Entomology, which was to be populated by researchers from the country's major cities. I helped Sehnal move his Prague lab to the South in his old Wartburg car. The station wagon, a pitiful product of East-German socialist design, ran despite glitches. On one trip, the gas pedal was getting stuck in the depressed position, so whenever Franta pushed the clutch to switch gears, I had to bend to the floor in synchrony and lift the gas pedal by hand. The wipers did not work either, so we would often stop to clean the windshield with snow, picked by the roadside.

Starting the lab in České Budějovice had glitches like those of Sehnal's Wartburg. Influenced by emerging trends, Sehnal envisioned building a laboratory of molecular insect science. However, unlike his superb expertise in developmental endocrinology, Sehnal's grasp of molecular methods was platonic. Michal Žurovec was the only among us skilled in molecular cloning. I still owe Michal for teaching me the vital basics. Sehnal's intuition told him what ought to be done, but he could not show us how. Supplies and instruments were in dreadful shortage (we washed our used yellow and blue tips and Eppendorf tubes). The solution was sending students around the world to more advanced labs, which is what we did after traveling was enabled by the collapse of the communist system in 1989. As soon as he managed, Sehnal himself left to work with Peter Bryant at the UC Irvine. Whoever visited Sehnal at Irvine had to bring back to our home lab loads of plasticware and reagents, even enzymes and radioactive labels on dry ice. Traveling was different then.

Lynn Riddiford at the University of Washington in Seattle was another of Franta's links, and I was privileged by Lynn's invitation to her lab. The plan was to clone *Galleria* homologs of nuclear receptor genes and characterize them as insect hormone receptors. Sehnal was aware of current progress and capable of procuring cDNA clones for genes such as *Drosophila E75* from Bill Segraves in the Hogness lab (Segraves and Hogness, 1990) and for human retinoic acid receptors from Pierre Chambon (Petkovich et al., 1987). In the early 1990′s it seemed plausible that receptors for lipophilic insect hormones would resemble their vertebrate counterparts and, therefore, could be found based on DNA sequence homology. Genomes were not sequenced at the time, so the cDNAs would be radio-labeled and used as hybridization probes to screen bacteriophage libraries prepared from insect DNA. After initial hiccups, *Galleria* orthologs of *E75* and *HR3* were cloned (Jindra et al., 1994a,b) and my Ph.D. was done. I continue to be grateful to Franta and Lynn for arranging my stay in the beautiful city of Seattle, an experience that has changed my life.

No matter what Sehnal did, he had a clear vision and rarely gave up. Hostile conditions could not stop him. He had a remarkable capacity to secure resources for whatever he intended to do, even if such resources seemed unavailable to every-one else. Have I explained yet how the word "sehnal" translates from Czech? It literally means "he who managed to get something (by chasing it down)". Pure coincidence.

## 3. Chasing too many rabbits?

A Web of Science search yielded 200 journal papers authored by Sehnal since 1966. He also wrote or co-authored some 15 books and book chapters, of which the impressive Growth and Life Cycles (Sehnal, 1985) was already mentioned. My favorite is the comprehensive chapter Evolution of Insect Metamorphosis (Sehnal et al., 1996) which, in my opinion, captures the essence of postembryonic insect development and surpasses other contemporary texts on metamorphosis. Sehnal and his colleagues uniquely combined morphological, phylogenetic, physiological, and endocrine aspects to convincingly support the Hinton theory on the homology of the holometabolous pupa with late hemimetabolous juveniles (Hinton, 1963) (see Bellés, 2020 for detailed analysis). The chapter by Sehnal et al. (1996) provided essential guidance for my own attempt to cover the subject (Jindra, 2019). I dedicated the 2019 review to František Sehnal "for his lucid insight into the evolution of insect development".

To see which other research areas Sehnal has contributed to, I sorted his papers by topic. Most (about 40 %) deal with diverse facets of insect developmental endocrinology with papers on ecdysteroids, neuropeptides, and primarily juvenile hormone (JH). The figure would rise to 50 % if papers examining the utility of juvenoids in insect control were added. Regulation of circadian rhythms, diapause, and various other aspects of physiology including stress or metabolism make up another 13 %. Almost 17 % of all Sehnal's papers focus on insect silk glands, silk composition, and/or potential use of the glands' products. Insect-derived proteinases, proteinase inhibitors, and engineering of insects (silkworms) themselves are subject of about 15 studies.

Practical application of science was always an important part of Sehnal's work. More than a quarter of his papers are dedicated to biorational pest control using insect growth regulators (IGRs, primarily endocrine disruptors) and *Bacillus thuringiensis* (*Bt*) toxins. Sehnal was very progressive and actively promoted use of transgenic, particularly *Bt* crops in Europe. Most of his late research aimed to examine environmental safety of GMO plants and their interaction with arthropods in the field.

As evident from the above, František Sehnal was interested in everything that concerned insects. One might argue that by covering many subjects, he spread too thin to achieve a major breakthrough as a scientist and that he could have used more focus. However, the great breadth of scope was Sehnal's big asset when it came to activities beyond research. He devoted much effort to service for the scientific community and educational institutions. I have already mentioned his proactive approach to changing the stubbornly negative policies towards GMO crops in Europe. Between 2004 and 2008, Sehnal led the committee of the International Congress of Entomology. He served in editorial boards of multiple journals and in grant evaluation panels.

František Sehnal, originally trained as a teacher, cared about education in our country. In 1990, as soon as the oppressive political system vanished, he became active in founding the University of South Bohemia (inaugurated in 1991), built next to the institutes of the Czech Academy of Sciences in České Budějovice. Sehnal served as the vice-president of the university for science and international affairs before holding director positions at the Academy institutes. He also served on the national accreditation committee for higher education.

František Sehnal was well aware that good research requires a constant influx of motivated and curious students. Himself a gifted teacher, he insisted that academic staff take active part in courses taught at the new university. This mutually beneficial symbiotic interaction works on our campus to this day. Sehnal's own successful graduates present the best evidence of his legacy. In most of the numerous research areas he pursued, he has established a school of thought, followed by some of his disciples. I find it amazing and admirable that despite the many executive duties, Franta remained active in research and managed to run his own lab until very late in his emeritus years.

## 4. A project finished 20 years late

In setting research goals including student assignments, František Sehnal was incurably optimistic. My Ph.D. project was not supposed to end by cloning the two ecdysone-induced *Galleria* genes mentioned above. It originally aimed to "*unveil the mechanism of juvenile hormone action through identifying the JH receptor gene and characterizing its protein product including ligand-binding assays with JH and synthetic juvenoids*". Sehnal mailed the two-page project assignment to me in 1990 while he stayed at Irvine. Given the time of its conception, the plan was remarkably specific and well thought-of. The experimental design relied on (what else) *Galleria* as a model and, with no genome sequences and genetic tools such as RNAi or CRISPR available at the time, it was hopelessly ambitious.

The project remained buried in a drawer for 20 years during which I completed two postdoctoral stays and started my own lab. Meanwhile, others struggled in the suicidal pursuit of finding JH receptors (JHRs). Here I want to highlight the effort of Jean-Philippe Charles whose postdoc work in the Riddiford lab explained an earlier error leading to a false JHR candidate (Charles et al., 1996; Riddiford, 2020). Jean-Philippe's knowledge and experience proved instrumental when I approached him to collaborate on another receptor candidate, Methoprene-tolerant (Met). *Drosophila* Met was originally discovered by Tom Wilson (Wilson and Fabian, 1986; Ashok et al., 1998) and initially tested for JH binding by Miura et al. (2005). We were encouraged by the results of RNAi knockdown showing that Met was required for JH to block metamorphosis in larvae and pupae of the beetle *Tribolium castaneum* (Konopova and Jindra, 2007). After several trips to Jean-Philippe's lab in Dijon and after finishing many bottles of Burgundy wine, we had the critical ligand-binding assays on wild-type and mutated Met proteins done and the nanomolar affinity determined (Charles et al., 2011).

The old Sehnal's project assignment text was unearthed in June 2010 as I searched documents in preparation for my professorship lecture. František attended the talk. I was thrilled to surprise him by showing our fresh JH binding data next to the scanned copy of his revived, typewritten instructions on how to get the JHR nailed. The slide headline read: "*20 years later, I am almost done!*" I was very happy I could use the stage to pay tribute to my former mentor back then when he was present, and I would like to pay tribute to him again now that he has left us.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

## Acknowledgments

I remain grateful to František Sehnal for his mentorship and for introducing me to international science. In this respect, I would like to thank Lynn Riddiford for agreeing to František's proposal to accept me in her lab. The JHR work would not have been successful without the hospitality and excellent collaboration of Jean-Philippe Charles. Finally, I thank all of my collaborators and students who graduated from my lab since 1999.

## References

Ashok, M., Turner, C., Wilson, T.G., 1998. Insect juvenile hormone resistance gene homology with the bHLH-PAS family of transcriptional regulators. Proc. Natl. Acad. Sci. U.S.A. 95 (6), 2761–2766.

Bellés, X., 2020. Insect Metamorphosis: From Natural History to Regulation of Development and Evolution. Elsevier Inc., London.

Charles, J.-P., Iwema, T., Epa, V.C., Takaki, K., Rynes, J., Jindra, M., 2011. Ligand-binding properties of a juvenile hormone receptor, Methoprene-tolerant. Proc. Natl. Acad. Sci. U.S.A. 108 (52), 21128–21133.

Charles, J.-P., Wojtasek, H., Lentz, A.J., Thomas, B.A., Bonning, B.C., Palli, S.R., Parker, A.G., Dorman, G., Hammock, B.D., Prestwich, G.D., Riddiford, L.M., 1996. Purification and reassessment of ligand binding by the recombinant, putative juvenile hormone receptor of the tobacco hornworm, *Manduca sexta*. Arch. Insect Biochem. Physiol. 31 (4), 371–393.

Hinton, H., 1963. The origin and function of the pupal stage. Proc. R. Ent. Soc. Lond. A 38, 77–85.

Jindra, M., 2019. Where did the pupa come from? The timing of juvenile hormone signalling supports homology between stages of hemimetabolous and holometabolous insects. Phil. Trans. R. Soc. B 374 (1783), 20190064.

Jindra, M., Sehnal, F., 1989. Larval growth, food consumption, and utilization of dietary protein and energy in *Galleria mellonella*. J. Insect Physiol. 35 (9), 719–724.

Jindra, M., Sehnal, F., 1990. Linkage between diet humidity, metabolic water production and heat dissipation in the larvae of *Galleria mellonella*. Insect Biochem. Mol. Biol. 20 (4), 389–395.

Jindra, M., Sehnal, F., Riddiford, L.M., 1994a. Isolation, characterization and developmental expression of the ecdysteroid-induced E75 gene of the wax moth *Galleria mellonella*. Eur. J. Biochem. 221 (2), 665–675.

Jindra, M., Sehnal, F., Riddiford, L.M., 1994b. Isolation and developmental expression of the ecdysteroid-induced GHR3 gene of the wax moth *Galleria mellonella*. Insect Biochem. Mol. Biol. 24 (8), 763–773.

Konopova, B., Jindra, M., 2007. Juvenile hormone resistance gene Methoprene-tolerant controls entry into metamorphosis in the beetle *Tribolium castaneum*. Proc. Natl. Acad. Sci. U.S.A. 104 (25), 10488–10493.

Miura, K., Oda, M., Makita, S., Chinzei, Y., 2005. Characterization of the *Drosophila* Methoprene-tolerant gene product. FEBS J. 272, 1169–1178.

Petkovich, M., Brand, N.J., Krust, A., Chambon, P., 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330 (6147), 444–450.

Riddiford, L.M., 2020. *Rhodnius*, golden oil, and Met: A history of juvenile hormone research. Front. Cell Dev. Biol. 8, 679.

Segraves, W.A., Hogness, D.S., 1990. The E75 ecdysone-inducible gene responsible for the 75B early puff in *Drosophila* encodes two new members of the steroid receptor superfamily. Genes Dev. 4 (2), 204–219.

Sehnal, F., 1985. Growth and Life Cycles. In: *Comprehensive Insect Physiology, Biochemistry and Pharmacology* (eds. G.A. Kerkut, L.I. Gilbert), Pergamon Press, Oxford, Vol. 2, pp. 1–86.

Sehnal F., Švácha P., Zrzavý J., 1996. Evolution of insect metamorphosis. In *Metamorphosis: Postembryonic Reprogramming of Gene Expression in Amphibian and Insect Cells* (eds. L.I. Gilbert, B.G. Atkinson, J. Tata), Academic Press, pp. 3–58.

Wilson, T.G., Fabian, J., 1986. A *Drosophila melanogaster* mutant resistant to a chemical analog of juvenile hormone. Dev. Biol. 118 (1), 190–201.